(PUBLISHED QUARTERLY)

Journal of the Council for Scientific and Industrial Research.

Vol. 5. MAY, 1932. No. 2.

Observations on the Resistance of Sheep to Infestation by the Stomach Worm (*Haemonchus contortus*).

By I. Clunies Ross, D.V.Sc.*

1. Introduction.

In a recent article, Stoll (1929) records the development in two lambs of a very marked degree of resistance to infestation with *Haemonchus contortus,* following an initial light experimental infestation in the case of one animal and subsequent continuous exposure to natural infestation on restricted pastures of both animals, as the result of eggs voided by the experimentally infested lamb. Initially, each showed evidence of a normal and progressively increasing infestation with *Haemonchus contortus,* as indicated by steadily rising egg production, which reached as much as 13,600 per gm. of faeces in one case, and 10,000 per gm. in the other after three months. After remaining at this peak for about one month, egg production rapidly declined, and ceased a few weeks later, four months after the first eggs of the parasite appeared in the faeces. Subsequent exposure of these animals, in the one case to heavy experimental infestation, and in the other to repeated risk of natural infestation on heavily contaminated pastures, failed to set up any appreciable degree of fresh infestation, it being found that larvae experimentally administered were rapidly voided in large numbers in the faeces.

On a post-mortem examination, both animals were found to have a very slight infestation with *H. contortus,* individual worms being smaller than normal, and showing evidence of poor egg production. Stoll concluded from the evidence thus obtained that *Haemonchus* infestation of sheep, provided it is not so heavy as to overwhelm the resistance of the animal and lead to its death but yet of sufficient severity to provoke an active immunological response, will result in the development of an immunity or resistance which may be absolute.

In view of the fact that our personal experience with *H. contortus* infestation in Australia, both in the field and under experimental conditions, presented many features at variance with these conclusions of Stoll, it was thought advisable to put forward the following observations in regard to this problem.

* Officer in Charge, F. D. McMaster Animal Health Laboratory, Division of Animal Health, C.S.I.R.

2. Field Observations.

Station A.

In the early summer of 1928, the writer had occasion to visit a large Queensland station, some 80,000 acres in area, on which the normal carrying capacity was about 40,000 sheep. This property was situated in an area noted for heavy infestation with *H. contortus,* and, to a lesser degree, with *Oesophagostomum columbianum.* In the course of investigations carried out at this station, a number of aged ewes which had been bred and run continuously on the property for four or more years were autopsied. Of eight thus examined, six showed degrees of infestation with *H. contortus,* varying from 400 to over 1,000 worms, while the other two were completely negative. A number of lambs, which were less than six months old, were also examined on the same property, and these all showed some hundreds of worms, the numbers being comparable to those found in the ewes examined, with the exception of the two negative cases. On this property, worm infestation is so heavy and serious that, until the introduction of a system of periodic drenching with carbon tetrachloride, in 1928, losses in the late summer and autumn amongst young sheep reached from 1,000 to 5,000 per annum, while lambing ewes also suffered considerable mortality.

In this case, therefore, it is seen that, though the ewes had been continuously exposed to recurring infestation for a number of years, the majority of those examined showed considerable degrees of infestation, such infestations being comparable to those of lambs suffering their first season's infestation. It should be mentioned also that these cases were examined early in December, whereas worm infestation becomes worse during the later summer months and early autumn, so that it might be expected that the animals would show even heavier infestations later in the season.

Station B.

This was a similar Queensland property, in the same district as Station A, but larger in extent (200,000 acres), and carrying normally about 70,000–80,000 sheep. Prior to 1928, it had been practically impossible to rear young sheep on this property, and at that time it was almost wholly stocked with aged wethers, which had been brought in from worm-free country further to the west. This station was re-visited in 1930, some years after breeding had been resumed. Ten aged "culled" wethers, from nine to eleven years old, which had been running on the property continuously for from four to six years, were autopsied. Eight showed from 500 to more than 1,000, but two less than 100 *H. contortus.* Two four-tooth sheep, three two-tooth sheep, and four lambs were also autopsied, and all showed moderate to heavy degrees of infestation with *H. contortus,* these infestations on the average being no heavier than those of the aged wethers.

Therefore, on these stations both ewes and wethers had been exposed to continued infestation over a number of years without any marked resistance (as determined by the degree of infestation), becoming evident, except in a minority of aged animals. This would suggest either—

1. That the sheep were subjected to repeated infestation which was not sufficiently heavy to call forth a strong degree of resistance—in the circumstances most unlikely; or

2. that any resistance developed was evanescent, and did not last from season to season; or
3. that the production of such resistance as Stoll describes is in no way constant, and possibly is determined by factors additional to those he suggests.

In addition to these observations in the field, the following experimental data were obtained in the laboratory.

3. Experimental Observations.

The following observations were made on individuals of four groups of sheep which previously had been exposed to artificial infestation with *H. contortus* in connexion with tests of the anthelmintic properties of certain drugs given in licks and in drinking water. It may be noted that none of these drugs appeared to have the slightest effect on the parasites, sheep in all lots that received such drugs showing as regular and heavy degrees of infestation, and suffering as much mortality as those in the untreated control group.

For the purpose of this paper, these sheep may be considered as re-grouped according to age and breed:—

Lot A.—Four ewes, three being aged (S 43, 44, and 45), and the other a four-tooth (S 78).

Lot B.—Six merino lambs eight to twelve months old (S 72-77).

Lot C.—Six Lincoln cross merino lambs eight to twelve months old (S 80 to 85).

Lot D.—Four merino lambs three months old (S 91-94).

All animals were kept in concrete-floored yards, which were swept and hosed out daily. Food, which was supplied in troughs, consisted of oaten chaff, crushed oats, and bran in the morning, and lucerne hay at night.

(i) *Degree of Worm Infestation at Commencement.*

At the beginning of the trial, all sheep in *Lot A* were found to have a heavy degree of infestation with *H. contortus,* faecal cultures showing over 90% of this species. One animal (S 43) showed marked pallor of the visible mucous membranes, while two others (S 44 and 78) showed definite, but less marked, signs of clinical haemonchosis, though the degree was not determined by erythrocyte or leucocyte counts.

Lot B.—All animals in this group were found by faecal culture to be heavily infested with *H. contortus,* cultures showing over 90% of larvae of this species. Egg counts of the faeces of each sheep by the Stoll method showed from 6,000 to 33,000 eggs per gm.

Lot C.—No animal in this group had a higher egg count than 900 to 2,000 eggs per gm., while larvae consisted of approximately equal numbers of *H. contortus* on the one hand, and *Ostertagia* spp. and *Trichostrongylus* spp. on the other. No animal showed any clinical signs of haemonchosis.

Lot D.—Repeated culture and egg counts of this group showed no evidence of infestation with *H. contortus* or other helminths, except *Strongyloides* spp. No animal showed any clinical signs of haemonchosis.

(ii) *Nature of Treatment Given.*

Lot B was treated with 1 cc. tetrachlorethylene per sheep on the 22nd December, 1930, and *Lots A* and *B* with 2 ccs. carbon tetrachloride on the 29th December, 1930. Subsequent faecal culture and egg counts showed a marked decrease in the degree of infestation with *H. contortus* in all animals in both these groups. In Lot A, all clinical evidence of haemonchosis disappeared in from two to three weeks, while in Lot B, cultures showed only from 5% to 32% *H. contortus* one month after treatment, and counts ranged from 600 to 4,000 eggs per gm. of faeces. Lot C continued to show only light infestation, and Lot D to be negative (except for *Strongyloides* spp.).

Artificial infestation of all lots was begun on the 13th February, 1931. Each sheep was drenched with larval cultures containing not less than 95% *H. contortus,* from 500 to 1,000 larvae being administered each week to Lots B, C, and D until the 22nd May, 1931, when a total of 6,500 larvae had been given to each animal. Larvae were administered to Lot A at the same rate as to Lots B, C, and D until the 16th April, 1931, after which from double to four times the number were given at each drenching, so that on 20th May, 1931, each of these sheep had received 11,000 larvae.

(iii) *Observed Effects of Infestation.*

From four to five weeks after artificial infestation was begun all sheep in Lots B, C, and D showed progressively increasing degrees of infestation, and by 8th April all sheep in these lots showed over 90% of *H. contortus* larvae in cultures, there being little difference in any of these groups. With Lot A, however, though there was a high percentage of *H. contortus* larvae in cultures, and though one sheep showed a count of up to 5,000 eggs per gm. from week to week, in no case was there evidence that any steady progressive increase in infestation was taking place. On the 27th April, 1931, S 75 (Lot B) died after suffering from intermittent diarrhœa for about ten days. This animal could not be autopsied for some 24 hours, by which time any *H. contortus* were dead and degenerated. It was not considered, however, that death was due to haemonchosis, though prior to death the visible mucus membranes were markedly anaemic.

At this time, some sheep in Lots B, C, and D were showing counts of up to 20,000 eggs per gm., and it was evident that very heavy infestations had been established. During the next three weeks, a number of clinical cases of haemonchosis began to develop in Lots B, C, and D, and in these lots nearly all animals showed counts of from 20,000 to 60,000 eggs per gm., but no sheep in Lot A (adults) showed any increase in infestation, and all made satisfactory gains in weight.

From thirteen to seventeen weeks after infestation was begun the following animals died or received anthelmintic treatment when showing marked symptoms of acute haemonchosis or other evidence (egg counts and larval cultures) of heavy infestation:—S 73, 74, 75, 76, 77, 81, 82, 83, 84, 85, 91, 92, 93, 94, all of which were in either Lot A, B, or C. Only three animals (S 73, 80, and 84) out of these three lots remained in apparently normal health up to the 18th week. S 84, however, though it showed no pallor of the mucus membranes, and appeared lively and in normal health, gave egg counts repeatedly reaching as high as 20,000 to 40,000 eggs per gm., over 95% of larvae in cultures being *H. contortus.* S 73, though also in good condition, was

shown by repeated egg counts (up to 33,000 eggs per gm.) to have an equally heavy infestation. In both these animals, therefore, in spite of their apparent resistance, infestations comparable in intensity to those in more obviously affected animals were set up. In the case of S 80, egg counts were as low as 2,600 to 4,800 eggs per gm., yet on autopsy in the 23rd week (the sheep being kept under the same conditions as during the trial) this animal was found to have from 500 to 1,000 *H. contortus* (exact number not counted)—a moderately heavy infestation. In this animal, the egg counts had remained surprisingly low, considering the degree of infestation found.

The routine examination of sheep was discontinued after 18th June, 1931 (eighteen weeks after infestation was begun), when faecal cultures of the adult sheep, S 78, 45, 43, and 44 (Lot A), were either negative (S 43 and 44) or showed very few *H. contortus* larvae (S 78, 45).

On autopsies between 18th to 24th August, 1931, these sheep contained the following *H. contortus*:—S 78, 4; S 45, 154; S 43, none; and S 44, none.

4. Discussion.

In the above observations on animals artificially infested with *H. contortus* it is seen that all four ewes (one four-tooth and three aged), which had been freed to some extent from an earlier and heavy infestation by medicinal treatment, subsequently proved refractory to attempts to re-infest them. A group of lambs (Lot B), also freed to some extent from heavy infestation, all proved as susceptible to re-infestation as two other lots (C and D), one of which had had a previous very light infestation, and the other no previous infestation.

On the other hand, in the natural infestations recorded, though the aged ewes and wethers examined had been exposed to infestation from year to year, only a small minority showed evidence of an absolute resistance to infestation, the majority still harbouring a considerable number of worms.

In contrast, we have such phenomena as the self cure and subsequent resistance to re-infestation on the part of lambs, as recorded by Stoll (loc. cit.), the marked individual resistance, as noted by ourselves, of adult sheep in lots experimentally infested with *H. contortus,* and, as in Stoll's cases, the complete self cure of heavily infested animals without treatment.

It would appear, therefore, that the degree of resistance to infestation with *H. contortus* is influenced by a complex of forces of the individual importance of which we are not yet aware. Among such forces, the following suggest themselves:—natural resistance, age resistance, an acquired resistance due to a prior infestation, and nutritional factors.

(i) *Natural Resistance.*

There is at present little experimental evidence that individual young sheep, not previously exposed to infestation, have any natural resistance to infestation, that is, a resistance inherent in the individual, and appearing in animals of the same age and breed maintained under identical conditions. Fourie (1931), however, endeavoured to infest lambs artificially with pure cultures of *H. contortus,* and though these lambs are stated never to have been exposed to infestation previously,

he found that in a group of 38 lambs nine fatal cases of haemonchosis were set up, six heavily infested animals recovered, while 23 resisted and did not show the effects of infestation. It is not stated whether the resistant 23 animals failed to become infested, or merely proved resistant to the effects of any infestation set up; but, since enormous numbers of larvae were administered (up to 50,000) in the cases recorded in detail, it would appear that the degree of resistance varied very greatly. (No egg counts of individual animals are given.) That this might be due in some cases, however, not to resistance to the actual infestation, but rather to resistance to its effects, is suggested by the case of S 73 and 84 above, which, though very heavily infested, showed no clinical evidence of haemonchosis.

We have recorded recently (Ross, 1931) that in two newly-born worm-free lambs, receiving 1,000 *H. contortus* larvae each, 48.5% and 6% of larvae developed respectively. But in this case the larvae were administered in a single dose to each lamb—a highly unnatural procedure—so that, having regard to the variable course which fluids may follow in reaching the abomasum when given to sheep by the mouth, it cannot be accepted that this variation reflected the true degree of resistance in the two animals. In connexion with hookworm infestation in the dog, it has been shown by Herrick (1928) that, even in puppies exposed to their first infestation, the number of larvae developing varies as widely as 6% to 60%.

There seems some ground, therefore, for believing that individual natural resistance may play an important part in determining the degree of infestation set up naturally or artificially in lambs.

(ii) *Age Resistance.*

Among stockmen and others it is a commonplace that aged sheep, with the possible exception of lambing ewes, suffer much less from the effects of worm infestation than lambs, it being frequently said that, once a sheep becomes a two-tooth (eighteen months old approximately), there is little likelihood that it will show marked effects of worm infestation. There is, however, little experimental evidence that such sheep are less susceptible to infestation than young lambs, though they certainly show less marked effects of such infestation. Again, under natural conditions, it is impossible to determine to what degree any resistance shown is due to age, and to what degree to the effects of a prior infestation. The great majority of sheep in Australia exposed to infestation when aged have already been infested in their youth. We have recorded, on the other hand, that aged animals killed in the field, as well as in Lot A above, may show considerable or even heavy degrees of infestation.

(iii) *Acquired Immunity.*

There appears little doubt that at least a temporary and partial immunity to infestation does frequently follow infestation, such resistance commonly being encountered where attempts are made experimentally to infest lambs which are not worm-free. In most instances, however, such resistance is not absolute, since light infestations are maintained throughout, or may be set up. Acquired resistance, if produced, must evidently be of short duration, at least in many cases, in view of the heavy infestations met with in sheep of all ages in areas of heavy enzootic infestation, though only the younger animals may exhibit obvious effects of parasitism.

McCoy (1931) has also found in the case of dogs that, though such resistance is developed in animals exposed to repeated infestation with *Ancylostoma caninum,* so that they may throw off heavy infestation and prove temporarily refractory to re-infestation, yet after a lapse of time, some may be re-infested once or even twice.

In the sheep, it is probable that a greater degree of resistance may be developed in older and stronger sheep than in lambs, as suggested by the variation in susceptibility to re-infestation of Lots A and B above.

The production of a strong and lasting acquired resistance to *H. contortus,* particularly if persistent, would be of interest, in view of the fact that, at the present time, the most striking examples of either age immunity, or acquired immunity to metazoon parasites are perhaps those in which there is a suggestion of abnormal host-parasite relationship, such as the age immunity to *Syngamus tracheae* in chickens (Ransom, 1921), and acquired immunity to the trematode infestation with *Nanophyses salminicola* (Donham, Simms, and Miller, 1926) in the dog. We have recently suggested (Ross, loc. cit.) that *H. contortus* is specifically adapted to survival in the sheep rather than the ox, and the host-parasite relationship with the former is the normal one. Were this so, it would perhaps be expected that either an age immunity or a prolonged and absolute acquired immunity would be less likely to be developed than if the host-parasite relationship were abnormal. This may be contrasted with the difficulty which Monnig (1926) found in infesting Merino sheep over two years old with *Trichostrongylus rugatus* and *T. instabilis,* while aged Persian sheep remained much more susceptible. Here, it is highly probable, is a difference in immunological response, due to the variation in the host-parasite relationship in the two breeeds.

It may be stated, therefore, that, though an acquired immunity to *H. contortus* not infrequently follows a heavy degree of infestation, such resistance, even in aged animals, may be of such temporary duration that the animals may subsequently be re-infested.

(iv) *Nutritional Factors and Resistance.*

It is very generally held that nutritional factors exercise a vital influence on the degree of infestation of sheep with *H. contortus.* Here again, however, resistance to infestation is commonly confused with resistance to the effects of infestation. Thus the fact is frequently cited that sheep which are apparently in normal health when feed is young and nutritive in the spring and early summer, develop marked haemonchosis when it dries off in the late summer, autumn, and even winter, and so becomes less nutritious. This, however, may be due, not to the fact that animals then become more susceptible to infestation, but either to the fact that infestation has been steadily built up throughout summer and autumn, and ultimately reaches a degree sufficient to produce symptoms of haemonchosis, or to the fact that animals in a poor nutritive condition show the effects of infestation more markedly than those infested to an equal degree, but adequately nourished. It is not improbable, however, that the acquired resistance of sheep may be weakened, or entirely dissipated, by their subjection to impaired nutritive conditions.

Foster and Cort (1931) have recently demonstrated experimentally that the acquired resistance of dogs to infestation with *Ancylostoma caninum* may be readily removed by placing them on deficient diet, and no less rapidly restored by an adequate diet. If the degree of susceptibility of sheep to *H. contortus* and the duration of acquired resistance are affected in a similar manner, many observed facts would be explicable, including the greater susceptibility of Lot B compared with Lot A (see above), since the nutritive requirements of young sheep being more exacting than those of aged animals, they might under field conditions obtain comparatiely less adequate nutrition, and the production of a degree of resistance be correspondingly impaired. In this also would lie the explanation of the frequent heavy infestation of aged sheep in areas of heavy enzootic infestation, especially under unfavorable seasonal conditions. There would possibly still be contradictions, such as the absolute immunity enjoyed by a minority of aged animals in such areas, but these might well be animals in which already a renewal of resistance has been acquired temporarily, or which exhibit an idiosyncratic natural resistance.

These facts have a very important bearing on questions of the control of worms in sheep, and particularly the safeguarding of young animals, since it is evident that aged animals may be heavily infested with *H. contortus,* although not necessarily exhibiting any effects of such infestation, and may thus serve seriously to contaminate pastures simultaneously or subsequently grazed over by young sheep.

5. References.

Donham, C. R., Simms, B. T., and Miller, F. W., 1926.—The so-called salmon poisoning in dogs. *Jour. Amer. Med. Vet. Ass.*, **68**: 701.

Foster, A. O., and Cort, W. W., 1931.—The effect of diet on hookworm infestation in dogs. *Science*, **73**: 681.

Herrick, C. A., 1928.—A quantitative study of infections with *Ancylostoma caninum* in dog. *Amer. Jour. Hyg.*, **8**: 125.

McCoy, O. R., 1931.—Immunity reaction of dog against hookworms (*Ancylostoma caninum*) under conditions of repeated infection. *Amer. Jour. Hyg.* **14**: 268-303.

Monnig, H. O., 1926.—The life-cycles of *Trichostrongylus instabilis* and *T. rugatus* of sheep in South Africa. 11th and 12th Rep. Div. Vet. Ed. & Res., Part 1, pp. 231-251.

Ransom, B. H., 1921.—The turkey an important factor in the spread of gapeworms. U.S. Dept. Agric. Bull., 939.

Ross, I. Clunies, 1931.—The host specificity of *Haemonchus contortus* of sheep and cattle. *Aust. Jour. Exper. Biol. & Med. Sci.*, **8**: 217.

Stoll, N. R., 1923.—An effective method of counting hookworm eggs in faeces. *Amer. Jour. Hyg.*, **3**: 59.

Stoll, N. R., 1929.—Studies on the strongyloid nematode *Haemonchus contortus*. *Amer. Jour. Hyg.*, **10**: 384.

Research on Bees: A Progress Report.

By G. A. Currie, B.Sc., B.Agr.Sc.*

A brief note in regard to the objects of the Council's investigations on bees, and in regard to the grant from the Rural Credits Development Fund that had been made available for the assistance of that work, was given in a previous issue (see this *Journal* 4 : 253, 1931). Attention was drawn in that note to the fact that the Australian bee industry is responsible for a production worth £100,000 per annum, based on official statistics alone, that Apiarists' Associations consider the industry annually produces very much more than that amount, as there are numbers of beekeepers holding relatively small areas from whom no statistical returns are obtained, and that the industry is apparently capable of considerable extension if certain disabilities, including dwindling, can be overcome. Although the onset of cold weather has now put an end to the study of certain phases of the investigations, some advances in the work have already been made. An account of these is given in the article that follows.—Ed.

Summary.

The problem of D.T. or pollen deficiency disease in Australia is being investigated by the Council in conjunction with the Victorian Apiarists Association. The problem seems to consist of finding an efficient substitute for pollen, to be given to the bees during a period of pollen shortage in the field. The research is divided into three parts—

(1) Laboratory.—Finding a substitute for pollen which will lead to the development of the brood food glands in the head of the young worker adult.

(2) Insectary.—Testing substitutes in the hive to see if they can develop the larvae during the period of progressive feeding to the adult stage.

(3) Testing substitutes in the field on a commercial scale during a period of pollen shortage.

The laboratory stage is fairly well advanced. Yeast, casein, and certain mixtures of these, with other substances, have been shown to develop the brood food glands.

The insectary work is showing indications of success, but field work has not yet been commenced, owing to the non-development of the trouble during the past two seasons.

1. Introduction.

In certain parts of Australia it has been observed that in dry years, generally during the months of January, February, and March, bee stocks have dwindled rapidly and died out, although honey has been plentiful. The areas concerned are chiefly those in which yellow-box, *Eucalyptus meliodora,* is one of the main honey-bearing trees. Mr. Beuhne† and other apiculturists have observed that under such conditions the pollen from yellowbox flowers and from certain other Eucalypts flowering at the same time is not available to the bees owing to its stickiness. Further, dry seasons prevent ground flora from flowering, and a condition arises in which no pollen for brood rearing is available to the bees, so that, as the old field workers die off from natural causes, no young ones are reared to take their places. This dwindling was called D.T. (Disappearing Trick), or deficiency disease.

* An officer of the Division of Economic Entomology, C.S.I.R.

† Late Apiculturist to the Victorian Department of Agriculture, and now Secretary, Victorian Apiarists Association.

Several endeavours have been made by various people in the past to have some research work initiated with a view to a control of the problem. The name of Mr. Tarleton Rayment comes readily to mind in this connexion. Further, in 1928, the New South Wales Apiarists Association and the Victorian Apiarists Association both passed resolutions desiring that research into this problem be undertaken. Two years later, the Victorian Association succeeded in having a grant from the Rural Credits Endowment Fund of the Commonwealth Bank made available to the C.S.I.R. for research into the problem.

Many methods of overcoming the pollen deficiency had previously been considered. Amongst these were—

(1) The introduction into the Australian flora of trees known to flower and produce pollen at the season of shortage.

(2) The transferring of pollen-filled combs from one district to another.

(3) The collection and storage of natural pollen during periods of abundance against periods of shortage.

(4) The cultivation of low growing plants for pollen production under irrigation.

All of these were rejected as impracticable. There only remained further researches into the possibilities of efficient pollen substitutes.

When C.S.I.R. took up the problem, it was decided that Mr. Beuhne's estimate of what the problem was, viz., one of pollen deficiency, should be accepted as the starting point of the investigation, and that the first definite aim should be to try out possible pollen substitutes exhaustively.

Arrangements were made for the field work to be undertaken by Mr. Beuhne, and the author was allowed to devote a part of his time to the laboratory work. The Council provides without charge the use of the laboratory, microscopes and other instruments, insectary space, and electric power and light.

2. Pollen Substitutes in General.

For many years, beekeepers all over the world have fed various substitutes for pollen to their bees in times of scarcity. Rye flour, wheat flour, pea flour, and Mellin's food are typical examples of the substitutes used, and it is well known that the bees readily collect and store such substances. However, critical tests have not borne out the claims made for these substances, and it appears unlikely that brood has ever been successfully reared to the adult stage wholly on these substitutes (Parker, 1926).* On the other hand, some evidence has been obtained in recent years that milk and sugar fed to bees in spring may lead to vigorous brood rearing when pollen is scarce (Winson, 1930)†. There are many sides to the use of pollen in the hive to be met by a substitute. Thus, to be successful, a substitute must be—

(1) A dry powder;

(2) not repellant to bees, so that they will collect and store it;

* Cornell Univ. Agr. Expt. Sta., Ithaca, N.Y., Memoir 98.

† *American Bee Journal*, September, 1930, p. 434.

(3) chemically constituted so that it will stimulate the brood food glands of the young adults to produce food for the queen, and for the young larvae during the period of mass feeding; and

(4) so constituted that, when fed mixed with honey to the larvae in the period of individual feeding it will allow them to develop to the fully functional adult stage.

Bees also use pollen in the cappings of the brood cells, but it is not certain if it is an esential constituent of these.

Dr. Soudek (1927)* of Czecho-Slovakia, has demonstrated that yeast and egg albumen can produce development of the brood food glands, but, in the state in which they are ordinarily procurable, they do not fulfil the other conditions of an ideal pollen substitute. After all the above conditions have been met, it must be remembered, too, that any pollen substitute to be used commercially must be cheap enough to warrant its use. Bees may use up to 7 oz. of pollen a day in a big colony at the height of brood rearing, but requirements normally would be much smaller than this.

3. Plan of Present Investigations.

The work now under review was planned out in the following sequence:—

(1) A check of the effect of known pollen substitutes and any new ones on the development of the brood food glands of young workers in an incubator.

(2) A test of these substitutes on a colony basis in an insectary where pollen is not available, to determine if brood can be reared to the adult stage.

(3) Following (1) and (2) above, a field test of any promising substitutes, the test to be carried out on a commercial scale and during a period of pollen deficiency.

When work was commenced, the first necessity was a supply of bees to draw from, so some hives of Carniolans were purchased late in 1931. From these, brood comb containing capped worker cells has been continually drawn. An incubator maintained at about 31° C. is used in the laboratory, the pieces of brood comb from which the young workers emerge being kept in gauze-topped glass jars. As the adults emerge, they are put into small boxes provided with wire-gauze covers, and the food for the bees is placed in pieces of comb, which are sealed to the bottom of the boxes. At first, the pollen substitute was mixed with sugar syrup and put into the cells, so that the bees were compelled to take it. Later, it was found that when a dry pollen substitute was used, it could be packed in the outer cells like pollen, and the syrup placed in the cells in the centre. Under these conditions, the young adults feed readily on the dry substitute.

* Bull. de l'Ecole Superieure d'Agronomie, Brno. R.C.S.

4. Development of Brood Food Glands.

Experimental Series 1.

In each case, 25 bees or more were used for a single experiment, and the results were as follows:—

Substitute.	Results.	Remarks.
Casein	No development	
Casein and yeast	No development	
Egg albumen	Slight development ..	Bees died off early
Egg albumen and yolk ..	Slight development ..	Bees died off early
Gelatin	No development	
Pollard of wheat	No development	
Pollen (Control)	Fair development	
Pea flour	No development	
Pea flour and yeast	Slight development	
Pollard and yeast	No development	
Syrup only (Control)	No development	
Yeast	Fair development	

Experimental Series 2.

Substitute.	Results.	Remarks.
Casein	Slight development	
Casein and yeast	Good development ..	Bees healthy and lived to 32 days
Casein and cystine	No development	
Casein and pea flour	No development	
Cystine	No development	
Cystine and pea flour	No development ..	Probably died from syrup becoming too concentrated
Cystine and peptone	No development	
Milk, dried (Trufood)	Fair development	
Milk and yeast	Fair development	
Peptone	No development ..	Bees died off very rapidly (within 2 days), except when solution of peptone in sugar syrup was very weak, when they lived up to 7 days
Pollard (wheat offal)	No development ..	Bees collected on their legs as if natural pollen
Syrup only	No development	
Yeast (dried and pulverized in mortar)	Fair development ..	Bees healthy and active

Experimental Series 3.

Substitute.	Results.	Remarks.
Casein 1 part, yeast 1 part ..	Fairly good development	
Casein 5 parts, yeast 1 part ..	Fairly good development	
Casein 10 parts, yeast 1 part ..	Fairly good development	
Casein 15 parts, yeast 1 part ..	No development	
Casein 20 parts, yeast 1 part ..	Fair development	
Milk (dried) 1 part, pea flour 2 parts	Fair development	
Pollard 8 parts, yeast 1 part ..	Fair development	

Experimental Series 4.

Substitute.	Results.	Remarks.
Casein only	Fair development	
Casein 25 parts, yeast 1 part ..	Fair development	
Casein 1 part, egg albumen, dry powder, 1 part	Slight development	
Casein 10 parts, dried milk 1 part, yeast 1 part, pollard 1 part	Fair development	
Egg albumen, fresh	Fair development	
Egg albumen, dry (powdered) ..	No development	
Egg albumen, dry (powdered), 1 part, dried milk 1 part	Fair development	

The results, as can be seen, are not regular, but the general conclusions are that yeast alone or in admixture with various cheaper protein foods can stimulate the brood food glands to active functioning.

Another most important finding is that casein alone can lead to development of the pharyngeal glands. This substance is cheap enough to warrant its use, and being a dry powder it is easily stored and fed to the bees. Probably this substance can be used to combine with yeast and pollard to give an effective substitute. Experiments with these substances are at present in progress.

When pollard was used it was noticed that the bees generally died off fairly early (about ten days old), and that they died with the abdomen distended with gases. It seems probable that substitutes containing much starch cause digestive disorders, as it is well known that the bee cannot digest starch.

From the point of view of cheapness it would be desirable to add as much pollard as possible to any mixture, but it would appear that the foods richer in protein and poorer in starch are more healthy for the bee. Further series of tests will be set out to determine the cheapest effective substitute.

5. Feeding of Larvae.

The larvae newly hatched from the egg are fed by the "nurse" bees with the "brood food" from the glands referred to above. This "mass feeding" is carried on for the first two and a half days of the larval life, when the young larvae float on the brood food and absorb it. When plenty of young adults are present (four to ten days old), and sufficient pollen for their feeding is present in the hive, the supply of brood food is lavish.

After the period of "mass feeding," the larvae enter the period of "progressive feeding," during which period they are fed by the very young adults on pollen mixed with honey and water.

After experiments with bees in the incubator had demonstrated that the brood food glands could be stimulated by pollen substitutes, the next step was to test out the substitute as a food for the larvae during the period of progressive feeding after the second day of life. In order to get a pollen-free area, a colony of bees was put into an enclosed space 30 feet x 10 feet in an insectary. The old bees soon

died off, because they could not adjust themselves to the small range, but the bees which hatched out in the insectary took to it kindly enough.

The queen ceased laying as soon as she was enclosed, but when the young bees started flying they were supplied with pollen substitute in artificial flowers, and a little desultory egg laying began. The bees stored the substitute (casein, yeast, and pollard) in the cells, and apparently reared some brood on it, as capped brood was seen shortly afterwards in one of the combs. The difficulty of being quite certain that no trace of pollen was present in any of the combs from which the young brood emerged added to the fact that the brood cappings eaten by the emerging adults are made up of a mixture containing pollen, make it necessary to repeat the experiment a number of times.

Another hive was made up of capped brood combs and placed in a compartment in the insectary. Sugar syrup was fed outside the hive for two and a half months, and no pollen substitute given, so that if any pollen existed in the hive it would be completely used up. No brood rearing took place during the period. On 20th February, 1932, a frame was filled with pollen substitute and placed in the hive. It was noticed that after the pollen substitute had been put in the hive the bees began to fly more freely than before. However, no brood rearing took place.

On 31st March, a piece of comb containing eggs, unsealed larvae, and a few sealed cells was cut from a comb in an outside hive. All traces of pollen were carefully removed, and the piece of comb fitted into a space in an empty brood comb. This was placed in the experimental hive in the insectary, and abundant dry pollen substitutes shaken into the empty cells surrounding the brood. This substitute was made up as follows:—

Pure casein	25 parts.
Yeast (dried)	1 part.
Pollard	6 parts.
Pea flour	1 part.
Dried milk	1 part.

The hive was then closed up for 16 days and sugar syrup fed to the bees. During these 16 days, all sealed brood would have hatched out, and all larvae which had reached the progressive feeding stage when put into the hive would have pupated and emerged adults. When the hive was opened after the 16 days, eggs and brood in all stages were present, the queen having been stimulated to lay by the presence of the other brood. All the brood present then was either the progeny of the queen of the experimental hive or else from eggs or larvae, under three days old, from the outside hive. All the brood had been reared in the experimental hive from at least the mass feeding stage or the egg stage, and had been fed by bees which had had no protein food except the pollen substitute for their own nutrition and for feeding the older larvae. A piece of comb containing sealed brood was cut out and placed in an incubator. Adults emerged from the 17th day onwards, all apparently healthy. A fresh cell capping removed on the 17th day disclosed a larva which pupated on the 19th day. This larva, which would probably have been from an egg laid about 11 days before, must have been laid by the queen in the experimental hive. The experiment is being continued to check the present indications, but meanwhile it can

be stated that bees have been reared from egg to adult on a pollen substitute.* Further experiments will be necessary as checks, and also to discover if the adults reared on substitutes are as efficient and as resistant to disease as normal adults.

6. Investigations in the Field.

Mr. Beuhne has kept in touch with the investigator on the one hand and with the apiarists on the other. If a pollen shortage occurs, it is hoped that he will be able to help in conducting experiments to try out the pollen substitutes under commercial conditions.

7. Acknowledgments.

The writer is indebted to Dr. R. J. Tillyard for constructive criticism in the preparation of the manuscript, and to Mr. M. R. Freney for information on chemical points in connexion with the research.

* American investigators have succeeded in rearing larvae on pollen substitutes, but none of these larvae, so far as we are aware, were able to pupate and emerge adult.

The Root System of the Sultana.*

By C. Barnard,† M.Sc.

Summary.

The observations, recorded here in respect to the Sultana vine in the Mildura district, have been made primarily for the purpose of supplying information which is required for investigations dealing with methods of cultivation and irrigation. It has been found that—

(1) The rooting habit of the Sultana is shallow and widespreading, and a considerable overlap of the root systems of adjacent vines planted 11 x 9 feet occurs, restricting the growth of the individuals.

(2) Sub-soiling prior to planting increases the depth at which the main roots normally develop, though neither subsequent drainage nor irrigation has much effect in this respect. The depth of penetration of the smaller roots, however, is controlled by drainage.

(3) Root pruning occasioned by winter ploughing to a depth of 9-10 inches is beneficial.

(4) The feeding roots are annual structures, invariably associated with an endophytic mycorrhiza, and are developed at a depth of 5-10 inches at the base of the cultivation zone.

(5) The root growth commences about five weeks after the rise of the sap begins and three weeks after bud-burst.

(6) The great mass of feeding roots present by the end of November are borne on new extension growth, which has arisen from the ascending laterals, decapitated during winter ploughing.

1. Introduction.

The importance of an exact knowledge of the root development of crop plants has become increasingly apparent during the last few years. The practice in the past has been to record the responses of a plant to any treatment by studying the reaction of the aerial portion only. It is now recognized, however, that an understanding of the reactions of the root system, which constitutes one-half of the individual plant, is often of paramount importance in planning experiments and interpreting the results. It is also of importance in determining sound methods of tillage as well as a scientific procedure in the application of irrigation water and fertilizers. Recent horticultural research in Australia into these questions has indicated the need for an investigation of the root development of our orchard trees. Such studies have been commenced, and the results obtained in respect to the sultana grape are presented in this article. The immediate objects of the inquiry were—

(*a*) to determine the normal type of permanent root system laid down under irrigation in the Mildura district;

(*b*) to investigate the seasonal development of the roots, with special reference to the reaction of the feeding rootlets to the cultural practices and system of periodic irrigation obtaining in that settlement.

---

* This investigation was carried out at the Commonwealth Research Station, Merbein, Victoria, during the years 1928 and 1929, while Mr. Barnard was an officer of that station.

† Botanist, Division of Plant Industry, C.S.I.R.

2. The Structure of the Root System.

The general structure of the vine root system may be regarded as analogous to that of the aerial portion of the plant, in that a number of main roots, which branch freely, form the framework of the system, and correspond to the branches. These roots are perennial, although growth ceases in winter and recommences in spring, the older portions becoming woody structures similar to the woody branches. On the youngest portions of these permanent roots, small absorbing or feeding rootlets are borne, and these, in a sense, correspond to the leaves. They are of limited growth, hardly ever being more than 1½ inches long, never becoming woody, and functioning for one season only. The root system of the sultana is thus composed of two types of roots, the permanent or extension and the temporary or feeding roots.

The main roots originate from the base of the trunk at a depth of 12 inches to 14 inches, and spread radially, sloping gently downwards. Their extent and degree of ramification depends principally on the age of the vine, though the number of main roots is not increased after the second or third year from planting. In the case of eight-year old vines, they normally attain a length of 9 to 12 feet, and at this distance have reached a depth of 18 to 20 inches.

The smaller permanent roots arising from the main root system show a marked tendency to rise towards the surface of the soil, though a few are developed below the level of the main roots between 18 inches and 30 inches, and occasionally "plunging" roots of this order penetrate to a depth of 4 feet.

The feeding roots are, for the most part, borne on the ascending laterals, and form a zone 5 inches to 10 inches below the surface of the soil, but they may rise even closer to the surface when not displaced by cultural operations.

3. Permanent Roots.

(*a*) *Horizontal Distribution.*—The framework, as stated above, spreads in a horizontal plane in all directions, the main roots averaging approximately 10 feet. Some, however, reach 15 or 16 feet, and an occasional one may attain a length of 24 feet. As the vines are planted 9 feet apart in rows 11 feet apart, the roots overlap considerably, and the growth of each is reduced by competition. The effect of this overlap has been shown by means of a comparison between the end vine of the row and the third one. The circumference of the butt at a level of 6 inches above the ground and the amount of annual wood removed at pruning were taken as a measure of vegetative development. The figures obtained in this study were as follows:—

	No. of Vines.	Mean Circumference of Trunk.	Difference.	Standard Error of Difference.	Probability.
Terminal vine	19	22·5 cm.	2·4 cm.	·65 cm.	·0001
Third vine	19	20·1 cm.	..	..	..
		Wt. of Prunings.			
Terminal vine	19	5·5 lb.	..	..	..
Third vine	19	4·3 lb.	1·2 lb.	·31 lb.	·00005

The greater vigour of the terminal vine is obviously due to the fact that the root system meets with no competition on one side.

A similar examination on a field in which the end vine had been planted at an average distance of 6 ft. 3 in. from the second vine, whilst the second was placed at the standard 9 feet from the third, gave results just as conclusive in the reverse direction. Eleven pairs showed that the mean circumference of the terminal vines was 17.1 cm., and that of the third vines 20.7 cm. The mean difference is 3.6 cm. The third vine is normal in size, and comparable to the third vine on other fields, but the closer spacing between the terminal and second vines had been more than sufficient to counter-balance the usual advantage enjoyed by the terminal plant.

Deep ploughing operations at the end of winter actually help to decrease this overlap, as the main roots are often cut. This results in the formation of strong new growth in spring within the area allotted to each vine, and is undoubtedly a beneficial practice. The laterals ascending from the main roots are also cut severely by the deep ploughing. New extension growth, however, arises from the cut ends, and, growing horizontally, forms a mass of new roots at the base of the cultivation zone. Bunches of feeding rootlets arise from these new roots during the same growing season, while cultivation to a depth of approximately 5 inches prevents the feeding zone from rising nearer to the surface of the soil.

(*b*) *Vertical Distribution.*—The vertical distribution of the roots is correlated with the soil profile in which three distinct horizons are found in the first 3 feet of soil. These are described below—

(i) The cultivated zone, which is divided into an upper tilth layer of approximately 5 inches and a lower layer representing the soil, which is disturbed only by deep ploughing. This second layer extends from 5 inches down to about 9 inches, and by reason of its slightly darker colour, is sharply defined from the horizon beneath.

(ii) A zone extending from 9 inches to a depth of approximately 16 inches, composed of the same soil as horizon (i) and differing mainly in possessing a much lower humus content.

(iii) Between 16 inches and 18 inches, horizons (ii) and (iii) merge into one another. Horizon (iii) is slightly heavier in texture than the overlying soil, and is generally characterized by the presence of leached nodules of lime, which give it a mottled or blotched appearance. This horizon extends to a varying depth, and becomes heavier below 36 inches.

The distribution of the roots in relation to the soil profile is illustrated in Figure 1. The main scaffolding roots lie at the junction of horizons (ii) and (iii), whilst the tendency of the smaller roots which arise from them is to ascend through horizon (ii) to the basal layer of horizon (i), where they bear large masses of feeding rootlets along their ultimate branches. The depth and extent of each of the three horizons varies throughout the district, and this variation is more important than any differences in the texture of the soil in determining the vertical distribution of the roots.

The distribution of the main roots is not modified by drainage subsequent to planting or by irrigation, but is markedly influenced

by the treatment of the soil prior to planting. Deep sub-soiling of the land before planting undoubtedly results in more deeply-seated main roots.

The small roots, however, respond to the influences of irrigation and drainage, the former limiting and the latter inducing deep penetration. Even moderate irrigation without sufficient drainage may limit the depth of penetration to within 24 inches of the surface. Natural or artificial drainage on the other hand induces the development of small roots in the sub-soil. In the case of natural drainage, the open texture of the sub-soil results in their more or less uniform distribution down to a depth of 3 feet, whilst in the case of artificially-drained land, though a general downward trend is manifest, it is only in the immediate vicinity of the drains that many roots occur at this depth.

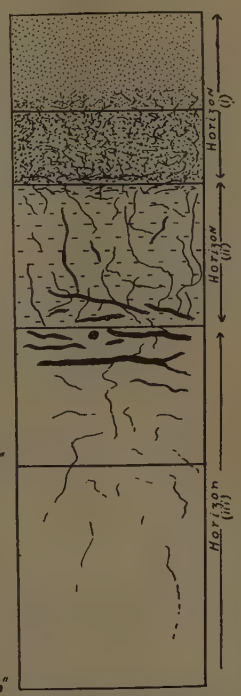


Fig. 1.—The soil profile and the vertical distribution of the roots.

It may safely be stated that the tendency for the majority of laterals to ascend towards the surface and the occurrence of a definite horizon of feeding rootlets at the base of the cultivation zone is not a feature induced by irrigation, the habit of surface feeding being natural to the vine.

4. Annual Growth.

The feeding roots of the sultana are sharply differentiated from the permanent or extension roots. They are of limited growth, and do not form any secondary tissues. They attain a length of ¾ inch to 1½ inches, and rarely branch. For the most part they occur on the current season's growth of the extension roots, and a new crop is produced annually. This type of feeding-root is quite different from the fibre type found in most orchard trees.

The ascent of the sap about the middle of August indicates the commencement of root activity, though new root growth does not appear until the third week in September. During this period, the only feeding roots present are a few of the last season's, the majority of which are decayed, brownish or black in colour, and devoid of any functional root hairs (1, Figure 2). In view of this fact, it is most remarkable that the flow of sap before the appearance of the new growth has been sufficient to bring about bud burst and initiate the early growth of the shoots.

The mechanism by means of which the vine is able to absorb moisture so rapidly during this period is not understood at present, but it seems probable that the endophytic mycorrhiza which is mentioned below, may play an important role in this connexion.

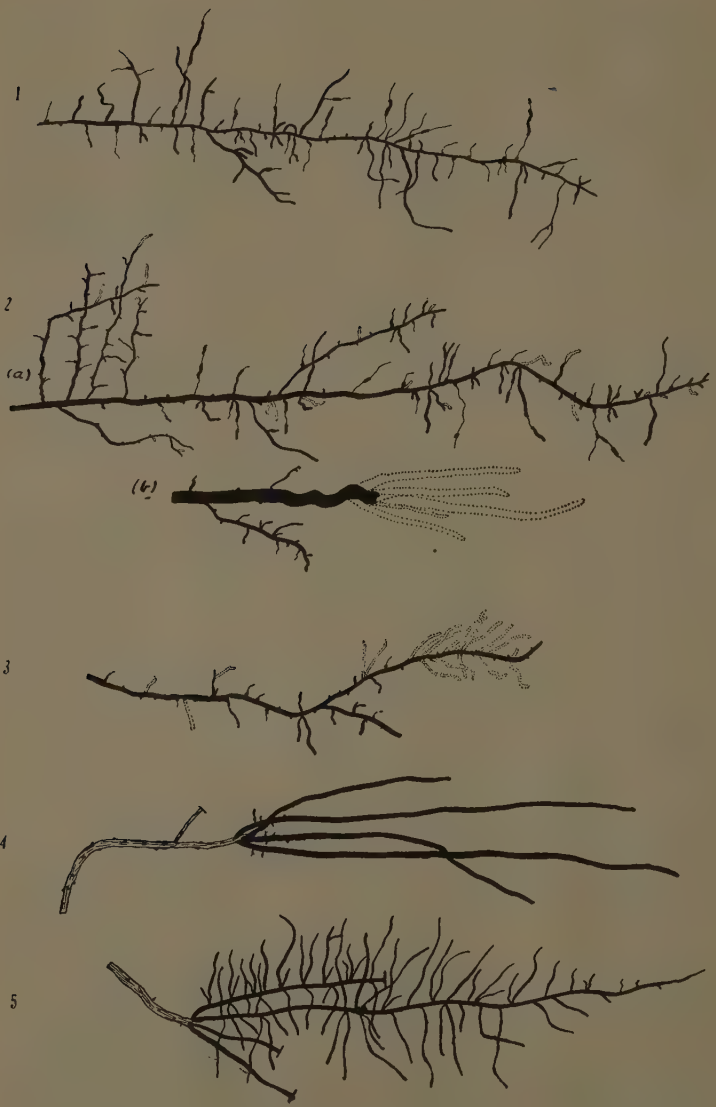


FIG. 2.—Growth during the season; about half natural size. (For explanation see text.)

Such a late development of new root growth has also been recorded in France,* but is contrary to the common belief that the bleeding of the vine is caused by the absorption of water by newly-formed roots.†

The first new growth consists of a few feeders arising on the previous season's elongation growth (2 (*a*), Figure 2) and extensions of the permanent roots (2 (*b*), Figure 2). The most marked development of the latter occurs near the surface of the soil, and originates from the ascending laterals, which have been decapitated during the winter ploughing operations. Two to six strong new extensions arise from the cut ends of such roots. The development of the new feeding rootlets is comparatively slow during October, and the stage reached towards the end of the month is illustrated in 3, Figure 2. About this time, new feeders appear at the base of the new elongation growth, which is shown in solid black in 4, Figure 2. The development of new extension root growth then becomes very rapid. By the third week in November these roots have reached lengths of 7 inches and 8 inches, and are covered with large numbers of feeder roots 5, Figure 2.

The young rootlets produce root hairs fairly abundantly early in the season, but by the end of November most of the feeders are mature, and there are very few rootlets with functional root hairs.

The effect of irrigation depends on the time of application, thus—

(*a*) Watering at the end of November results in the formation of white tips on the extension of growth and on some feeding rootlets, as well as in the formation of a few feeding rootlets on older roots. The average extension arising as a direct result of this irrigation was about 2 inches. The new growth soon turned brown, and growth apparently slowed down again approximately three weeks after the application of water.

(*b*) Watering at the beginning of January has the same effects, but the extensions to the permanent roots average only 1 inch. Later irrigations have a less marked and more temporary effect.

An endophytic mycorrhiza is invariably associated with the mature rootlets and a preliminary study of its development through the season has been made. It would appear that though at first the fungus is parasitic, the rootlets quickly react to its presence, and digestion of the hyphae to a certain extent takes place. The role played by the mycorrhiza in the nutrition of the vine is a subject, however, which requires further investigation.

5. Acknowledgments.

The author wishes to thank Dr. B. T. Dickson, Chief of the Division of Plant Industry, C.S.I.R., and Professor T. G. B. Osborn, of the University of Sydney, for helpful advice during the course of these studies. He is also indebted to Mr. A. V. Lyon, M.Sc.Agr., Officer-in-Charge, Commonwealth Research Station, Merbein, for assistance during the investigations.

* L. Rives—Recherches sur Quelques Formes de Deperissements de la Vigne, Court nous etc. Toulouse, 1926.

† A. I. Perolt—"A Treatise of Viticulture," MacMillan & Co., London, 1927.

The Chemical Treatment of Baits for Attracting Blowflies. I.

By Martin R. Freney, B.Sc.*

(Published on the recommendation of the Joint Blowfly Committee.†)

In submitting the following paper by Mr. Freney for publication, the Joint Blowfly Committee realizes that in ordinary circumstances it would have been preferable to await the results of further experimental work. The present strike season for Canberra is practically at an end, however, and several months must elapse before similar experiments can be conducted there. In the warmer districts, nevertheless, it will be possible for pastoralists to test the new baits on a large scale, and information regarding the results observed will be greatly appreciated. Although the pure form of sodium sulphide was used by Mr. Freney for obvious reasons, in all probability the commercial product, which is comparatively cheap, will prove equally satisfactory.

Not only will pastoralists who are endeavouring to cope with the blowfly pest by means of trapping be greatly interested in these experiments, but investigators of the problem as it occurs in other parts of the world will be equally interested. In Europe and North America, the latter will have opportunities of continuing or initiating experiments along similar lines during their strike season which is now approaching.

For these reasons publication is considered advisable at the present juncture.

J. A. GILRUTH,
Chairman of Joint Blowfly Committee.

In an earlier paper (this *Journal* **5**: 28, 1932), it was shown that a mixture attractive to primary blowflies could be obtained by hydrolysing keratin by means of sodium sulphide. This work has now been extended to include other substances which are rich in protein. Among these have been fresh sheep's liver and "blowfly soup," two of the baits most commonly used by pastoralists in blowfly traps. The present paper deals with the results obtained by treating these two baits with sodium sulphide.

Experiment 1.

Set up 8th February, terminated 4th March, 1932. Two small traps designed by Dr. A. J. Nicholson for use in experimental work were used.

Trap A was baited with 50 grams of fresh sheep's liver, to which 20 c.cs. of water was added.

Trap B was also baited with 50 grams of fresh sheep's liver and 20 c.cs. of water in which 5 grams of hydrated crystalline sodium sulphide was dissolved, i.e., a 20 per cent. solution.

Each day flies were removed from the bait-pan of both traps, and water was added to replace that lost by evaporation. The flies caught were killed, counted, and classified daily.

Trap A caught significant numbers of flies on the 1st to the 4th, and on the 7th to the 10th days of exposure. From the 10th to the 25th day, fourteen flies were caught. Numerous maggots developed in this bait, and by the 9th day the solid bait had become almost completely liquefied. The catches obtained in Trap B showed some fluctuations, but maintained a fairly steady average rate right to the end of the experiment. Very few maggots matured in this bait.

* Biochemist, Division of Economic Entomology.

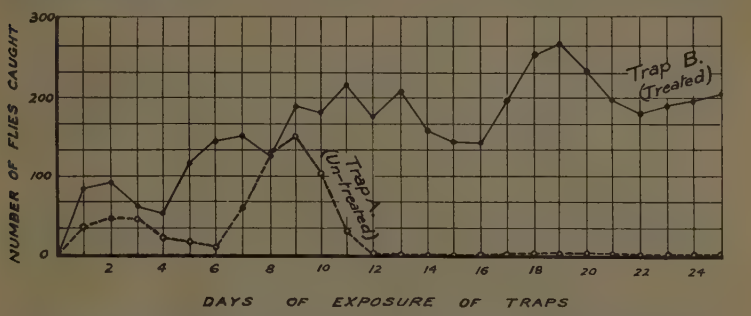
† Composed of representatives of the Council and of the New South Wales Department of Agriculture. (See this *Journal*, February, 1932, page 28.)

The results of the experiment are shown in the following table and graph:—

TABLE 1.

	Number of Flies. First 9 days' exposure.				Number of Flies. Total 25 days' exposure.			
	Trap A. Untreated.		Trap B. Treated.		Trap A. Untreated.		Trap B. Treated.	
Primary Flies—	females.	males.	females.	males.	females.	males.	females.	males.
L. cuprina	20	3	17	18	20	3	60	45
L. sericata	1	..	3	1	1	..	9	6
C. stygia	18	..	18	..	18	..	37	4
C. augur	459	19	918	31	460	19	2,358	186
Secondary Flies—								
Ch. rufifacies ..	61	5	265	18	64	5	683	184
M. varipes ..	53		30		55		343	
Sarcophaga spp. ..	8		22		16		93	
Primary Green Flies ..	24	3·7%	39	2·9%	24	3·6%	120	3·0%
Primary Brown Flies ..	496	76·7%	967	72·1%	497	75·2%	2,585	64·5%
Secondary Flies ..	127	19·6%	335	25·0%	140	21·2%	1,303	32·5%
Total	647	100%	1,341	100%	661	100%	4,008	100%

NOTE.—Primary Green Flies are *Lucilia cuprina* and *Lucilia sericata*. Primary Brown Flies are *Calliphora stygia* and *Calliphora augur*. Secondary Flies are *Chrysomyia rufifacies*, *Microcalliphora varipes* and *Sarcophaga* spp.



Graph 1. Daily catch of blowflies. Broken line Trap A (untreated bait); continuous line Trap B (treated bait). The points have been determined by a three point method of moving averages to show clearly the general trend of the catches.

This experiment showed that—

(i) the period of attractiveness of the sulphide-treated bait was much longer than that of the untreated bait;

(ii) the sulphide-treated bait was more attractive to all species of blowflies than the untreated bait; and

(iii) the increased attractiveness due to treatment was somewhat greater for secondary flies than for primary flies.

A similar experiment using Western Australian traps and larger baits was set up on the 4th March. Cold and rainy weather interfered considerably with this experiment, and the results were not conclusive, but again the treated traps caught a larger number of flies and remained attractive for a longer period than the untreated traps.

Experiment 2.

Set up 29th February, terminated 24th March. "Blowfly soup" was prepared by allowing trapped blowflies to putrefy in water in a Meteor trap for five days. The soup was stirred and divided into two

portions, each of three litres. Standard Western Australian traps* were baited with these, and to one was added 50 grams of crystalline sodium sulphide. Water was added from time to time to make up for that lost by evaporation. Despite unfavorable weather during the latter part of the experiment, the results were well defined and are shown in Table 2. For comparison, the analysis of a week's catch in Miss Fuller's seasonal record Western Australian trap (which is baited with 1,000 grams of fresh liver) is shown in the first column.

TABLE 2.

—	Fresh Liver.	Blowfly Soup.							
	1st March to 8th March, 1932.	8 days—29th February to 8th March, 1932.				24 days—29th February to 24th March, 1932.			
	Control.	Untreated.		Treated.		Untreated.		Treated.	
Primary Flies—		females.	males.	females.	males.	females.	males.	females.	males.
L. cuprina ..	50	4	6	80	..	4	6	85	..
L. sericata ..	45	2	..	20	15	2	..	40	15
C. stygia ..	450	61	29	520	125	62	29	1,090	140
C. augur ..	5,200	1,046	87	4,460	590	1,050	89	6,060	830
Secondary Flies—									
Ch. rufifacies ..	5,500	61	45	10,800	4,170	64	46	12,110	4,590
M. varipes ..	4,350	140		9,800		148		11,490	
Sarcophaga spp.	150	324		530		444		810	
Primary Green Flies ..	95	0·6%	12	0·6%	125	0·5%	12	0·6%	140	0·4%
Primary Brown Flies ..	5,650	36·0%	1,223	67·5%	5,695	18·4%	1,234	63·2%	8,120	21·8%
Secondary Flies ..	10,000	63·4%	570	31·9%	25,300	81·1%	706	36·2%	29,000	77·8%
Total ..	15,745	100%	1,805	100%	31,120	100%	1,952	100%	37,210	100%

This experiment showed that—

(i) the period of effective attractiveness of the sulphide-treated bait was much longer than that of the untreated bait, the former catching 15,000 flies and the latter 300 flies between the 4th and 24th days of the experiment;

(ii) the untreated bait was much less attractive than either fresh liver or the treated blowfly soup;

(iii) the treated bait was as attractive for primary flies, and more than twice as attractive for secondary flies as the fresh liver during the first eight-day period;

(iv) the total effectiveness of the treated bait was nineteen times that of the untreated bait; and

(v) treatment increased the attractiveness of the bait for all blowflies, but more for secondaries than for primaries.

Discussion.

Although the work is in an early stage, two important facts have been demonstrated and are worth recording. In the first place. it has been shown that the attractiveness of the bait to all species of blowflies can be increased by the sulphide treatment. This applies to the period in which the untreated bait is also attractive, i.e., to the first eight to ten days of exposure under the weather conditions prevailing at the time of the experiments. Thus, during this period, the ratio of the blowflies

* Meteor traps are not satisfactory for this work because the flies attracted cannot be counted or classified accurately.

caught by the treated bait to those caught by the untreated bait was 2:1 in Experiment 1 and 16:1 in Experiment 2. Most remarkable was the increase in the attractiveness of the blowfly soup. This bait has many advantages for practical use, but it has been shown in this laboratory that its attractiveness is distinctly lower than that of fresh liver. It now appears probable that this fault can be overcome by chemical treatment.

The increase in attractiveness is in general greater for secondary flies than for primary flies. This may be a disadvantage, but it is not proposed here to discuss its significance. Certain observations, however, suggest that this may be overcome by further treatment of the bait.

The second effect of the sulphide treatment is to prolong the period of attractiveness of the bait. This effect was more marked in Experiment 1 than in later work, and some observations made by Dr. Nicholson suggest that this prolongation is likely to be more pronounced in hot weather than in cold. How far it is due to direct chemical effects on the bait and how far to the deleterious effect on the sulphide on the maggots, which would otherwise devour the bait, has not been determined. The significance of this observation is that it opens up the possibility of less frequent baiting of traps than is at present practicable, a matter of considerable importance to those graziers who use blowfly traps.

The combined result of the increased attractiveness and the lengthened period of attractiveness is greatly to increase the efficacy of traps in which treated baits are used. Thus, the ratio of flies caught with the treated bait to those caught with the untreated bait over the whole period of the experiments was:—Experiment 1, primaries 5:1, all blowflies 6:1; Experiment 2, primaries 6.6:1, all blowflies 19:1. These results are in striking contrast with those obtained by Laake, Parman, Bishopp, and Roark* using potassium sulphide. These authors, however, do not give sufficient information to enable their results to be criticized.

In these experiments, certain arbitrary strengths of the hydrated crystalline sodium sulphide, which contains approximately 30 per cent. of the anhydrous sulphide, were used. Further observations are being made on the effect of the fused sulphide, which is both cheaper and stronger than the crystalline form. So far, these have indicated that high concentrations are not satisfactory.

To summarize, progress may be reported in the improvement of baits by chemical treatment, but further work is required at a favorable time of the year to determine—

(i) the correct amount of sodium sulphide to use under different conditions;
(ii) whether other chemical substances will produce a similar effect;
(iii) the effective duration of attractiveness of treated baits in warm weather; and
(iv) whether the attractiveness of the treated baits for primary flies can be increased.

The author is indebted to Miss M. Fuller and to Dr. A. J. Nicholson for permission to discuss unpublished work, and to Dr. I. M. Mackerras for help and advice in interpreting the results.

* U.S. Dept. of Agric. Technical Bulletin 270, November, 1931.

Caseous Lymphadenitis:

Ingestion as a Method of Infection of Sheep with the Preisz-Nocard Bacillus.

By H. R. Carne, B.V.Sc.*

(From the Pathology Department, F. D. McMaster Animal Health Laboratory.)

1. Introduction.

Although in Australia caseous lymphadenitis is generally believed to be contracted by sheep as a result of the entrance of Preisz-Nocard bacilli through wounds of the skin, certain of the earlier investigators of this disease were of the opinion that infection was most commonly due to the ingestion of food material contaminated with the causal bacillus. Carré and Bigoteau (1908) investigated a disease of sheep in France which in their opinion was due to an infection by the Priesz-Nocard bacillus, with subsequent toxaemia due to the production of toxin by these bacilli, which were restricted to limited foci of infection in lymphatic glands. Small purulent foci were found in the pharyngeal and retropharyngeal lymphatic glands in natural cases of the disease, and in these foci, pure infections of the Preisz-Nocard bacillus were demonstrated. These investigators considered that the usual portal of entry of the causal microbe is the alimentary canal, by way of slight injuries to the buccal mucosa, the organism being carried to the nearest lymphatic gland and there producing its lesions. A condition comparable with this acute intoxication by the Preisz-Nocard bacillus has never been reported in Australia, and abattoir statistics have shown that infection of lymph glands draining any part of the alimentary canal is rare. Gilruth (1902), who made extensive investigations of the occurrence of this disease in New Zealand, stated that pharyngeal, parotid, or sublingual lymphatic glands were never seen affected.

Cramp (1929) has recently made detailed post-mortem examinations of 2,378 sheep. Submaxillary lymphatic glands were found affected in only two cases, and pharyngeals in one. Extensive unpublished observations by the Meat Export Branch of the Commonwealth Department of Markets† have provided ample confirmation of the rarity of primary lesions associated with the alimentary canal.

A number of experiments in which sheep and also small experimental animals have been drenched with either caseous material from natural lesions, or with cultures of the Priesz-Nocard bacillus, have been reported.

Norgaard and Mohler (1899) gave to sheep food mixed with caseous material from natural lesions. No lesions were found on slaughter 49 days later. Seddon (1929) drenched three sheep with culture, and two with pus. Of those which received culture, two developed lesions in submaxillary, retropharyngeal, mediastinal, and prescapular lymphatic glands (after 60 days), and one of those which received pus developed lesions in submaxillary and retropharyngeal glands. The three sheep

* Lecturer in Pathology and Bacteriology, University of Sydney.

† Now Department of Commerce.

that became affected all showed lesions in the lymph glands of the head, and it appeared that the lesions present in other parts of the body were secondary to these. No lesions were present in the abdomen or thorax.

Carne and Clunies Ross (1931) drenched seven young sheep with massive doses of culture on two, and in some cases three, occasions. When killed from 37 to 51 days after drenching, three were found to have young lesions of caseous lymphadenitis in the submaxillary lymphatic glands.

So common is this disease in sheep that all infection experiments with sheep are open to the serious criticism that one has no definite proof that lesions found on post-mortem examination did not exist prior to the experiment. If care is taken, however, to use only young animals from flocks in which it is known that incidence of the disease is very low, significance can be placed upon the finding of lesions, particularly if these are examined histologically, as it is possible in this way to distinguish a young from an old lesion. This can be done with considerable accuracy even by the naked eye.

Although the behaviour of one species of animal cannot be presumed from that experimentally demonstrated in another species, the use of guinea-pigs for drenching experiments with the Preisz-Nocard bacillus has the advantages that this micro-organism produces a similar, though more severe, disease to that which occurs in the sheep, and, further the guinea-pig, while being quite susceptible to experimental infection by various routes, is not found naturally affected with the disease, as is the sheep. Drenching experiments can therefore be carried out under much more satisfactory conditions.

Norgaard and Mohler (1899) fed guinea-pigs with diets contaminated with culture. Death took place in five to eight weeks with lesions in the lymph glands draining the mouth and throat, and also later in other parts of the body. (Rabbits developed similar lesions after feeding culture with food, and Gilruth (1902) reported a positive infection by way of the alimentary canal in a rabbit). Recently, Dickinson and Bull (1931) have reported the results of drenching 99 guinea-pigs with culture. Of these, 59 animals became affected. Approximately half of the affected animals showed involvement of the cervical or submaxillary lymphatic glands. The distribution of lesions in some of the animals was of particular interest. In three animals, involvement was confined to the superficial lymph glands. Mediastinal lymphatic glands were only affected in three animals. From this experiment it is clear that, in some guinea-pigs at least, involvement of the superficial lymphatic glands may occur without infection being introduced through external injuries of the tissues drained by these glands.

We have been struck by the fact that, in both sheep and guinea-pigs, which have become infected as the result of drenching, lesions of the alimentary canal and its associated lymph glands, i.e., caudal to the pharynx, are rare. In sheep, when infection occurs as the result of drenching or feeding, the experimental evidence indicates that the portal of infection is the mucous membrane of the mouth or pharynx, and quite a large proportion of guinea-pigs show lesions of the lymph glands draining these cavities. In the buccal cavity, injuries to the mucous membrane may be caused by eruption of teeth, scratches by rough forage, &c., and such injuries act as portals of entry for the

causal bacilli in the same way as do injuries to the skin. It appears that, provided the Preisz-Nocard bacillus gains entrance to the buccal cavity, infection may take place via the buccal mucosa.

It is known that the lower part of the alimentary canal—stomach, small and large intestines—are by no means exempt from traumatic injury in the sheep, particularly by parasitic worms which are so constantly present, and frequently in large numbers. It would appear, therefore, that injuries of the gastric and intestinal mucosae are not lacking, and thus potential portals of entry for the Preisz-Nocard bacillus are numerous, yet in contrast with the findings in the buccal cavity, both naturally infected animals and animals which have been experimentally drenched, fail to show lesions of the lymph glands draining this part of the alimentary canal. We have shown (Carne and Clunies Ross, 1931) that even though widespread injury to the large and small intestine is produced by experimental infestation with large numbers of *Oesophagostomum columbianum,* and then massive repeated doses of culture of Preisz-Nocard bacilli are administered by drenching, thus providing a far greater concentration of the infective agent in the ingesta than could ever occur under natural conditions, yet even then lesions of caseous lymphadenitis of the stomach and intestines or their associated lymphatic glands did not occur except in one doubtful case, which may have been a secondary lesion.

As the result of these observations, we have been led to consider the possibility of the gastric secretion having a destructive action on the Preisz-Nocard bacillus which reaches the abomasum. On the supposition that the gastric juice might possess such an injurious effect, the following experiments were carried out to ascertain the result of introducing cultures of this organism per rectum.

2. Ingestion Experiments with Sheep.

Experiment 1.—A sheep was starved overnight, and the next morning given a purgative drench of magnesium sulphate. The animal was then starved, but allowed water for 24 hours, in order to allow the alimentary canal to be emptied as completely as possible. The rectum was finally washed out with copious enemas of water. By means of a sling around the loins, the hind-quarters of the animal were raised well above the level of the head, so that any fluid introduced per rectum would gravitate forward in the intestine. A rubber tube was then passed 3 feet along the colon, and through it was administered the combined cultures of Preisz-Nocard bacilli from seven agar slopes. The animal was kept in this position for twenty minutes, and then set free. This procedure was repeated in three days' time. Three weeks later, this sheep was killed, and no lesions of caseous lymphadenitis could be found throughout the body.

Experiment 2.—This was a repetition of Exp. 1, only in this case the sheep was not killed till six weeks after culture was administered, and *post-mortem* examination included culturing material from the lymphatic glands draining the intestines. No lesions of caseous lymphadenitis were detected, and cultural examination of lymph glands was negative.

The following experiments were designed to ascertain whether the filtered gastric juice of the sheep had any demonstrable injurious effect on the Preisz-Nocard bacillus.

Experiment 3.—The abomasal contents of four sheep were collected in clean bottles immediately the animals were opened up after slaughtering. Bottles were then packed in freezing mixture, and brought straight to the laboratory. This occupied approximately half an hour. Each sample was then filtered through paper, and finally filtered through either a Seitz EK special filter pad, or a Chamberland L3 candle. Some of each filtrate was then sown on serum broth to test for sterility. (All proved subsequently to be sterile). Of each remaining filtrate, 5.0 ccs. was introduced into a sterile test-tube, and placed in the incubator at 37° C. in order to bring it to blood heat. To each was then added two standard loopfuls of a 48-hour serum broth culture of Preisz-Nocard bacilli, which had been rendered as free from clumps as possible. Tubes were then agitated and replaced in the incubator. Subsequently, two standard loopfuls were removed and spread over the surface of serum agar plates at the end of the following periods after initial introduction of culture:—10 minutes, 25 minutes, 50 minutes, 1 hour 15 minutes, 1 hour 25 minutes, 1 hour 35 minutes, 1 hour 45 minutes, 2 hours, and 2½ hours. It was not considered necessary to continue observations over longer periods, as 2½ hours is about the maximum period that food would stay in the abomasum.

A control series of plates was run to each sample of gastric juice, consisting of a similar quantity of saline, into which were introduced two standard loopfuls of culture.

The results are shown in Table 1.

TABLE 1.

Time in Minutes.	Sample A.		Sample B.		Sample C.		Sample D.	
	Gastric Juice. Approx. No. of Colonies.	Saline Control. Approx. No. of Colonies.	Gastric Juice. Approx. No. of Colonies.	Saline Control. Approx. No. of Colonies.	Gastric Juice. Approx. No. of Colonies.	Saline Control. Approx. No. of Colonies.	Gastric Juice. Approx. No. of Colonies.	Saline Control. Approx. No. of Colonies.
10	500	400	500	500	400	20*	400	200
25	500	500	150	400	400	400	500	300
50	500	500	150	500	150	100*	500	500
75	150	500	50	500	30	500	500	500
85	500	500	50	500	8	400	500	500
95	500	500	22	300	12	400	500	400
105	500	500	17	500	2	500	500	500
120	500	500	5	500	5	400	500	500
150	500	400	2	400	0	400	500	500

* Plates badly spread, resulting in confluent colonies, which could not be accurately counted or estimated.

This experiment showed in samples B and C a definite decrease in the number of colonies on the plates sown with organisms which had been exposed to the action of gastric juice as compared with those which had been in saline under the same conditions. No significant difference occurred between samples A and D and their respective controls.

A second experiment of the same design was carried out, using a large collective sample of gastric juice from over 20 sheep instead of separate samples from individual sheep. The results of this experiment, however, showed no significant difference between the number of colonies

which developed on test and control plates. Because of our inability to confirm the suggestive results obtained in Exp. 3, the results of this experiment must be regarded with reserve.

The experimental observations available show quite definitely that sheep can be infected by ingestion or drenching of Preisz-Nocard bacilli. Such experimental administration of virulent organisms leads in most cases to infection of the lymphatic glands draining the buccal cavity, infection of the more caudal portions of the alimentary canal being rare. Since post-mortem evidence at the abattoirs shows that infections of lymphatic glands both of the gastro-intestinal tract and of the buccal cavity are very exceptional in sheep becoming infected under natural conditions, this would indicate that infection by ingestion, though possible, is not a common method of infection in sheep under natural conditions.

The experimental evidence suggests that Preisz-Nocard bacilli which reach the lumen of the intestine either by traversing the abomasum or by introduction per rectum, do not find suitable conditions there for penetrating into the tissues of the host to produce their characteristic lesions.

3. References.

Carne, H. R., and Clunies Ross, I. 1931. The association of the Bacillus of Preisz-Nocard with lesions caused by *Oesophagostomum columbianum* in sheep. *Jour. Coun. Sci. Indus. Res., Aust.*, 4: 78.

Carré, H., and Bigoteau, L. 1908. Le bacille de Preisz-Nocard en pathologie ovine—Sa toxine et les affections qui lui sont dues. *Rev. gen. de méd. vét.*, 17: 433.

Cramp, R. C. 1929. Observations on organ incidence, &c., of lesions of caseous lymphadenitis in sheep. N.S.W. Department of Agriculture. Veterinary Research Rept. No. 4, p. 32.

Dickinson, C. G., and Bull, L. B. 1931. Studies on infection by and resistance to the Priesz-Nocard Bacillus. (2) Susceptibility of the guinea-pig and the distribution of lesions after cutaneous and subcutaneous inoculation and ingestion. *Australian Jour. Exp. Biol. and Med. Sci.*, 8: 83.

Gilruth, J. A. 1902. Pseudo-tuberculosis in sheep. *Jour. Comp. Path. and Therap.*, 15: 324.

Norgaard and Mohler. 1899. The nature, cause, and economic importance of ovine caseous lymphadenitis. 16th Ann. Rep. U.S.A. Bureau of Animal Industry, p. 638.

Seddon, H. R. 1929. A discussion on the method of infection by the bacillus of Preisz-Nocard. N.S.W. Department of Agriculture. Veterinary Research Rept. No. 4, p. 38.

Caseous Lymphadenitis: The Growth of the Preisz-Nocard Bacillus in Sheep Faeces.

By H. R. Carne,§ B.V.Sc.

(*From the Pathology Department, F. D. McMaster Animal Health Laboratory.*)

1. Introduction.

The natural method or methods by which sheep contract infection by the Preisz-Nocard bacillus has naturally attracted special attention in the investigation of caseous lymphadenitis. Extensive detailed post-mortem observations on the distribution of the lesions of this disease have been made by veterinary officers of the Meat Export Branch of the Commonwealth Department of Markets, and by members of the veterinary staffs of the State Abattoirs (e.g., R. C. Cramp (1929).* These observations have emphasized the fact that the great majority of lesions are found in lymphatic glands draining the superficial parts of the body, whereas primary lesions of the alimentary canal are rare. Lesions are sometimes found in internal organs, not uncommonly in the lungs, and less frequently in the liver, kidneys and spleen, but such visceral lesions are metastatic infections, resulting from transportation of the causal bacilli by the lymph stream from affected superficial lymphatic glands to the lungs, and from there extensions may occur by organisms escaping into the general circulation, leading to their eventual arrest in various visceral organs, which appear to be predilection seats for the bacilli.

The preponderance of lesions in superficial lymphatic glands, together with clinical evidence collected in the field, strongly supports the view that the common natural method of infection is by the causal bacillus gaining entrance to the superficial parts of the body via wounds of the skin. The rarity of primary lesions of the alimentary canal suggests that infection via the alimentary canal is at least not a common natural method. The fact that primary lesions of the alimentary canal are not observed, however, does not exclude the possibility of infection taking place by this route. Experiments with the causal bacillus of glanders of horses have shown that at times when organisms were administered in sterile boluses from which the glanders bacilli were not liberated until disintegration had resulted from the action of gastric juice, primary lesions were observed in the lungs, and no evidence of any injury to the alimentary canal could be detected, the glanders bacilli apparently being able to penetrate through the bowel wall, possibly enclosed within phagocytic cells, without injury to the alimentary canal.

Carré and Bigoteau,† working in France, have expressed the opinion that ingestion of contaminated foodstuffs is a common method of infection. This opinion appeared to be based largely on the observation that the Preisz-Nocard bacillus was able to multiply readily in sterilized sheep faeces. Although the evidence provided by the post-mortem observations on the distribution of lesions in naturally infected animals, and the negative results following drenching of cultures to sheep, have been

* New South Wales Department of Agriculture, Veterinary Research, Report No. 4, p. 32 (1929).

§ Lecturer in Veterinary Pathology and Bacteriology, University of Sydney.

† *Rev. gén. de méd. vét.*, **17**: 433, 1908.

recorded by several investigators, Dickinson and Bull‡ have recently shown that drenching guinea-pigs with cultures of the Priesz-Nocard bacillus may apparently be followed at times by the development of lesions of the superficial lymphatic glands, without any observable lesions in the alimentary canal, or the lymph glands which drain it.

2. The Growth of the Preisz-Nocard Bacillus in Sheep Faeces.

If the Preisz-Nocard bacillus were able to grow in sheep faeces, as claimed by Carré, it appeared that such an abundant medium would serve as an excellent means of propagation of the organism outside the animal body, as it will grow quite readily at ordinary room temperatures on artificial culture media. Furthermore, if this organism were able to grow readily in sheep faeces outside the animal body, it might also be able to grow even more readily in the contents of the alimentary canal. If such a state of affairs existed, the numerous injuries to the mucous membrane of the bowel wall, caused by the worm parasites to which the sheep is susceptible, and with which it is so commonly infested, would provide the necessary portals of entry for the organisms into the tissues.

Confirmation of Carré's observation was therefore sought by carrying out the following experiments:—

A. *Sterilized Sheep Faeces.*

Experiment 1.—A sample of sheep faeces was collected at random from the experimental sheep pens. This was rubbed up in a mortar with tap water till of a soupy consistence. This liquid was then tubed and autoclaved at 120° C. It was found that a loopful of broth culture of the Preisz-Nocard bacillus seeded into this culture medium and incubated at 37° C. grew readily, so that after 48 hours incubation, examination of a loopful of culture revealed several thousand bacilli in each microscopic field. The point was not satisfactorily settled by this simple initial experiment, however. On mentioning this favourable result to two of our colleagues, we were surprised that they had carried out similar experiments without obtaining growth. On repeating the experiment ourselves, we found on this second occasion no evidence of multiplication of the organisms used for seeding, though these had still remained alive.

The following experiment threw some light on these negative results:—

Experiment 2.—Three lots of sheep, (*a*), (*b*) and (*c*), containing six, four, and six animals respectively, were fed on the following diets for a period of one week:—

(*a*) Green field peas.

(*b*) Oaten chaff and bran.

(*c*) Green grass growing around the Laboratory (composed of couch (*Cynodon dactylon*), 60 per cent., clovers and medics, 20 per cent.; *Poa annua*, 15 per cent.; *Paspalum dilatatum* and *Taraxacum densleonis* together constituting 5 per cent.).

‡ *Aust. Jour. Expt. Biol. Med. Sci.*, **8**: 83, 1931.

At the end of this period, a collective sample of faeces was taken from each lot. The hydrogen-ion concentration of each collective sample was determined and found to be as follows:—

(*a*) p*H* = 6.6.
(*b*) pH = 6.5.
(*c*) pH = 7.3.

A portion of each sample was then rubbed up with sufficient distilled water to reduce it to the consistence of thick porridge. This was poured in layers about 1.0 cm. deep in Petri dishes, which were then sterilized by autoclaving at 120° C. for 30 minutes, and subsequently found to be sterile.

Petri dishes prepared from each sample were then inoculated with a 1.10 dilution in physiological saline of a broth culture of Preisz-Nocard bacilli, which had been rapidly centrifuged for several minutes to throw down the larger clumps. A small depression was made in the centre of each dish by pressing down the medium with the end of a sterile glass rod. Into these depressions was placed one loopful of the diluted culture. By cultural tests of serial dilutions on serum agar plates, it was ascertained that this loopful of seeding material contained at least 42,000 organisms. The Petri dishes were then incubated at 37° C. After seven days, distinct small greyish colonies were seen to develop around the site of inoculation in several dishes containing faeces from animals fed on green peas. These proved to be pure colonies of Preisz-Nocard bacilli.

A loopful of faeces was taken from each plate at three points approximately 2.5 cms. from the site of inoculation, and also three more loopfuls from the margin of the plate, and all were sown into tubes of serum broth. In two of the dishes containing faeces from sheep fed on green field peas, a pure culture of Preisz-Nocard bacilli was obtained from material taken from points 2.5 cms. from the site of inoculation, and also from the margin of the culture (dishes were 9 cms. in diameter). Smears made from the faeces at the edge of the plates in these dishes showed approximately 200 organisms per microscope field.

In all plates except those containing faeces from sheep fed on green field peas, no evidence of growth was obtained, either by cultural methods or by direct microscopical examination of the faecal culture medium itself in smears.

This experiment indicated that the nature of the foodstuff on which the sheep has been nourished may play an important part in determining whether the sterile faeces will be a suitable culture medium for the organism. It should be noted that the amount of liquid used as seeding material was too small to account for spread of the organisms to the edges of the culture medium by simple carriage in streams set up by capillarity, and though a large number of organisms was present in the inoculum, there was definite increase in these as evidenced by the large numbers of organisms present in smears of material from various points round the margin of the plates.

Experiment 3.—Five sheep were fed for fourteen days on green field peas. A collective sample of faeces was used to prepare petri dishes of sterilized faeces as in Exp. 2. A plate was then sown with a loopful of dilution of broth culture of Preisz-Nocard bacilli, which contained the following numbers of organisms as determined by the plating out

method:—(*a*) 450, (*b*) 265, (*c*) 120, (*d*) 33, and (*e*) 2. (*Note.*—These counts can only be taken as a very approximate indication of the number of individual bacteria present in seeding material because of the characteristic spontaneous clumping of the organisms in cultures.)

Plates sown with all dilutions showed visible colonies at the site of inoculation from four to seven days after seeding. Portions of faeces removed from the margins of the plates, and sown on serum broth eight days and fifteen days after seeding in no case showed the presence of Preisz-Nocard bacilli.

In this experiment, although visible growth took place on the surface of the medium at the site of seeding, apparently no extension of growth through the substance of the medium to the margin took place.

Experiment 4.—Similar material was used to that employed in the preceding experiment, but instead of being placed in petri dishes, which were sown on the surface, the faeces were sterilized in test-tubes, and then seeded with the same dilutions of culture as used in Exp. 3. When examined after eight days incubation at 37° C., a definite marked increase was found to have occurred in all tubes, as determined by the method of plating out dilutions.

Experiment 5.—Faeces were collected separately from three sheep which had been fed on green cow-peas for fourteen days. This experiment was a repetition of Exp. 4, only a different lot of sheep was used, and the faeces from individual sheep were tested separately. Plates were incubated for eight days. In no plate did visible colonies appear, nor did the organisms appear to have grown through the substance of the medium, as portions of the medium taken from margins of the plates failed to show the presence of bacteria when sown in serum broth. Definite multiplication occurred, however, in tubes of mixed faeces from these three sheep sown with the same dilutions of culture at the same time as the plates. This may be explained by the greater quantity of water present in the tubes, the plates being somewhat drier than earlier batches.

Experiment 6.—Faeces were collected separately from two sheep which had grazed on green grass growing in a small paddock in the Laboratory. This grass was of the same composition as in Exp. 2c, only there was a young shoot after recent rains. Definite visible surface colonies developed five days after seeding in plates from both sheep. No growth through the substance of the medium in these plates was demonstrable.

Experiment 7.—Freshly voided faeces were collected from sheep which had been grazing on a pasture composed almost exclusively of subterranean clover. Plates and tubes were prepared, and sown with serial dilutions of culture. No surface growth was observed, but moderate growth occurred in the tubes as demonstrated by plating out on serum agar.

Experiment 8.—One sheep was fed on lucerne hay for ten days. Plates and tubes were prepared from faeces, and sown with serial dilutions of culture. Surface colonies developed on the plates, and definite growth occurred in the tubes, multiplication again being controlled by plating out on serum agar.

Experiment 9.—This was a repetition of 8, but a different sheep was substituted. Growth occurred both as visible surface colonies, and through the substance of the medium of the plates, and definite multiplication, controlled by plating out, was demonstrated in tubes.

Experiment 10.—Faeces from sheep fed on cow-peas were rubbed up in a mortar with tap water to form a thin porridge. This was then filtered through paper, and the filtrate passed through a Seitz EK filter pad. The filtrate thus obtained was found to be sterile. It was then tubed and seeded with dilutions of broth cultures of Preisz-Nocard bacilli. No growth took place after incubation at 37° C. for 20 days.

Discussion.—These experiments prove conclusively that the Preisz-Nocard bacillus will grow in sterilized sheep faeces under certain conditions. It was thought at first that a diet of green leguminous plants was necessary to provide the required conditions in the faeces to support growth, but subsequently it was found that, besides green cow-peas and green subterranean clover, a diet of green grass proved a satisfactory one. The definite multiplication which took place in faeces from sheep fed on lucerne hay showed that it was not essential for the diet to be in the green state. Our experiments on the determination of the hydrogen-ion concentration of the faeces on different diets, though somewhat limited, have not indicated that the pH is the limiting factor. It is thought that possibly the protein content of the diet may be related to subsequent growth in faeces, animals on a diet high in protein allowing sufficient to pass out unabsorbed in the faeces to support the growth of the Preisz-Nocard bacillus. From the fact that seventeen different sheep were used in these experiments, and group results were always the same, it does not appear that the suitability of the faeces to support growth is an inherent quality of certain animals, but does definitely depend on the nature of the diet.

B. *Sterilized Faeces in Competition with other Bacteria.*

It must be pointed out that, though the results of the foregoing experiments are very suggestive, it cannot be safely concluded that such a multiplication of the Preisz-Nocard bacillus will occur in unsterilized faeces under natural conditions. Faeces contain a large number of bacteria of various kinds, which are particularly adapted to growth in such a medium, so that a rather slow-growing organism such as the Preisz-Nocard bacillus will have very great competition for the available nutriment in such faeces, and will run the risk of being overgrown by other bacteria such as *Bacillus coli*.

Up to the present, all our attempts to isolate the Preisz-Nocard bacillus from natural unsterilized sheep faeces have failed. Even though freshly voided faeces be experimentally contaminated with large quantities of Preisz-Nocard bacilli, we have been unsuccessful in recovering the bacillus again, owing to the rapid overgrowth by other faecal organisms. It has also been found that, if faecal material suspected or known to contain Preisz-Nocard bacilli is inoculated in small amounts into guinea-pigs, the latter succumb to infections by organisms normally present in faeces before any lesions can be produced by the Preisz-Nocard bacillus.

Although we have not been successful in carrying out experiments with unsterilized faeces, the following experiments were undertaken to ascertain whether the Preisz-Nocard bacillus could grow in sterilized faeces in competition with two common faecal bacteria, namely, *Bacillus coli* and *Staphylococcus pyogenes aureus*.

Experiment 1.—A collective sample of faeces was taken from three sheep which had been fed on green field peas for fourteen days. A

simultaneous experiment showed that definite multiplication of the Preisz-Nocard bacillus occurred in this sample after sterilization, and seeding with the Preisz-Nocard bacillus alone.

After sterilization, plates and tubes were prepared in the usual manner. A surface depression was made in the centre of each plate, with a sterile glass rod, and into this depression was then introduced dilutions of culture of the Preisz-Nocard bacillus and *Bacillus coli* in varying proportions. The tubes of faeces were sown in the same manner, and both series were incubated for eight days at 37° C. Plates were examined daily for surface growth, but none was observed throughout the period of observation. On the eighth day, portions of faeces at varying distances from the centre of the plates were examined microscopically, and also sown on serum agar.

Table 1 shows the various proportions of culture of each organism used for sowing, and the results.

Table 1.

Number of Plate or Tube.	Sown with—		Results.	
	Preisz-Nocard Bacillus 2 loops.	*Bacillus coli* 2 loops.	Plates.	Tubes.
	Dilution of Culture.	Dilution of Culture.		
1	1 : 1,000	1 : 1,000	*B. coli* ..	*B. coli* and P.N. bacillus
2	1 : 1,000	1 : 10,000	*B. coli* ..	*B. coli*
3	1 : 1,000	1 : 100,000	*B. coli* ..	*B. coli*
4	1 : 10,000	1 : 1,000	*B. coli* ..	*B. coli*
5	1 : 10,000	1 : 10,000	*B. coli* ..	*B. coli*
6	1 : 10,000	1 : 100,000	*B. coli* ..	*B. coli*
7	1 : 100,000	1 : 1,000	*B. coli* ..	*B. coli* and P.N. bacillus
8	1 : 100,000	1 : 10,000	*B. coli* ..	*B. coli* and P.N. bacillus
9	1 : 100,000	1 : 100,000	*B. coli* ..	*B. coli*
10	1 : 1,000,000	1 : 1,000	*B. coli* ..	*B. coli*
11	1 : 1,000,000	1 : 10,000	*B. coli* ..	*B. coli*
12	1 : 1,000,000	1 : 100,000	*B. coli* ..	*B. coli*

In the plates, *Bacillus coli* alone was demonstrated by direct microscopical examination of smears made from faeces at various distances right out to margin from centre of plates. This organism was also recovered in pure culture from the margins of plates. In no instance was the Preisz-Nocard demonstrated in any plate.

In three tubes (No. 1, 7 and 8), a few small clumps of Preisz-Nocard bacillus were demonstrated microscopically in smears made from the faeces, but attempts to recover this organism were unsuccessful in every tube, a pure culture of *Bacillus coli* resulting in every instance.

Experiment 2.—This was carried out in a similar manner to the preceding experiment, the same sample of faeces being used, and both experiments were run simultaneously. Tube cultures only were sown, plates being omitted.

The results are seen in Table 2.

TABLE 2.

Number of Tube.	Preisz-Nocard Bacillus 2 loops. Dilution of Culture.	*Staphylococcus pyogenes aureus* 2 loops. Dilution of Culture.	Results.
1	1 : 1,000	1 : 1,000	*Staphylococcus aureus* and P.N. bacillus
2	1 : 1,000	1 : 10,000	*Staphylococcus aureus* and P.N. bacillus
3	1 : 1,000	1 : 1,000,000	No growth
4	1 : 10,000	1 : 1,000	No growth
5	1 : 10,000	1 : 10,000	No growth
6	1 : 10,000	1 : 100,000	*Staphylococcus aureus*
7	1 : 100,000	1 : 1,000	*Staphylococcus aureus*
8	1 : 100,000	1 : 10,000	*Staphylococcus aureus* and P.N. bacillus
9	1 : 100,000	1 : 100,000	*Staphylococcus aureus*
10	1 : 1,000,000	1 : 1,000	*Staphylococcus aureus* and P.N. bacillus
11	1 : 1,000,000	1 : 10,000	*Staphylococcus aureus*
12	1 : 1,000,000	1 : 100,000	*Staphylococcus aureus* and P.N. bacillus

In this experiment, it is seen that growth of the Preisz-Nocard bacillus did take place in the presence of *Staphylococcus pyogenes aureus,* but growth was rather scanty, and, as seen, irregular.

The first of these two experiments indicates that the overgrowth of the Preisz-Nocard bacillus in unsterilized faeces is a definite possibility; the second experiment shows that the Preisz-Nocard bacillus is able to maintain itself in the presence of at least one of the common bacteria of sheep faeces.

3. Conclusion.

In view of the fact that growth occurs readily in certain types of sterilized faeces, it is our opinion that the possibility of the Preisz-Nocard bacillus leading an external saprophytic existence should still be seriously entertained, until such time as definite proof of its behaviour in this external medium in its natural unsterilized condition is forthcoming, and until we have proof to the contrary, we consider that every precaution should be taken to minimize the risk of infection of sheep from accumulations of manure, particularly in positions to which sunlight does not have direct access. We have proof from experiments to be published shortly that the Preisz-Nocard bacillus is able to survive in sterilized sheep faeces which are protected from direct sunlight for over a year. This fact, quite apart from the possibility of its growth in faeces, warrants definite steps being taken to prevent the accumulation of sheep faeces in such places as shearing sheds, counting out pens, covered holding yards, and the like.

The Production of Tannin Extract from the Kino-impregnated Bark of Marri (*Eucalyptus calophylla*).

By W. E. Cohen, B.Sc.*

In previous issues of the *Journal* (e.g., 1 : 285, 1928 ; 2 : 161, 1929) references have been made to the investigations carried out in co-operation with the Forests Department of Western Australia and with the University of Western Australia with a view to the development of a satisfactory process for the commercial production of tannin extract from certain products of the forest. In the last issue (page 169) reference was also made to the commercial exploitation of the results of these investigations in so far as they related to karri. Before it was closed down, the tannin extract plant was used to study the possibilities of the production of extract from the kino-impregnated bark of marri. An account of that work is given below.—Ed.

Summary.

Recent investigations have shown that, by a simple process, an extract of satisfactory quality can be made from kino-impregnated marri bark.

Previous investigations involved severe and expensive treatment in order to obtain a high yield of tannin free from insoluble matter, and the processes employed caused considerable darkening in colour which naturally affected the value and quality of the extract.

From a consideration of the relationship between temperature of extraction and yield of tannin and of "insolubles," it has been shown that a satisfactory yield of tannin is attainable at 60° C. Above this temperature, no considerable increase in tannin is obtained, but a substantial increase in the yield of sparingly-soluble matter occurs. The latter introduces difficulties with respect to clarification, solubilization, and the colour imparted to hide substance.

A survey of a large number of solubilizing or dispersing agents has shown that sodium bisulphite is the most suitable material. By extracting kino-impregnated bark at 60° C. and subsequently treating the infusions with sodium bisulphite at 100° C. and evaporating, a very satisfactory liquid extract has been made in a plant test. This extract has been used to produce leather which has been reported to be of good quality and to be readily saleable.

1. Introduction.

Marri, or Western Australian redgum (*Eucalyptus calophylla*), grows very abundantly in the south-west portion of Western Australia. The forest is approximately 350 miles long by 50 miles broad at the northern end, widening to 200 miles in the south. The tree occurs mixed with jarrah (*E. marginata*) and karri (*E. diversicolor*), but patches of pure stands of marri are quite frequent. Hence the collection of bark for the production of tannin extract can be carried out under conditions conducive to low costs. The timber has at present little or no commercial value on account of the extent and frequency of gum veins and pockets.

It should be noted that marri bark, except when impregnated with kino, is very low in tannin content, but that, where the kino-exudations occur, the bark becomes a valuable source of tannin. It is possible to collect large quantities of the kino-impregnated bark with a high percentage of tans, and

* Senior Chemist, Division of Forest Products, C.S.I.R.

this would form a substantial source of tannin extract if the problems of solution and decolorization were satisfactorily solved on a commercial basis. It will be necessary, however, to purchase bark on the basis of its tannin content, and, with respect to this, every care will have to be taken in the collection and preparation of samples because of the variations resulting from the irregular occurrence of kino-exudations.

Under the direction of the former Institute of Science and Industry and, more recently, at the Brunswick Laboratory of the Commonwealth Council for Scientific and Industrial Research, a large amount of experimental work on the production of a suitable extract was carried out by Salt† and Coghill.‡ In addition to these investigations, a number of attempts to commercialize marri kino as a tanning material have been made from time to time by commercial interests.

Some attempts at tapping kino-impregnated trees are recorded in the Annual Reports of the Western Australian Forests Department. Details of quantities available are recorded by Salt* and Coghill.†

2. A General Discussion of the Problem.

When kino-impregnated marri bark is leached with hot water and the resulting infusion strained to remove small particles of bark, &c., a clear hot liquor, which when cooled below 50° C. becomes exceedingly turbid, is obtained. On analysis, this liquor is found to contain cold-insoluble matter amounting to as much as 20 per cent. of the total hot-soluble matter present. In addition, the colour it imparts to hide substance is of a dirty brownish nature, and is not desirable in leather manufacture. Hence, with marri extract, the main problems are—

(*a*) the elimination of matter insoluble in the cold either by removal or solubilization,

(*b*) the removal or brightening of colour of those bodies which give rise to the dirty brown colouration on hide substance.

In the past, the solution of these problems has been attempted by autoclaving, at temperatures of 100° C. and higher, either picked kino or extract (prepared by leaching the kino or impregnated bark with hot water and evaporating the extract), with solutions of solubilizing agents such as the acid and normal sulphites of the alkalies or mixtures of these. This procedure was claimed to have been successful in part because solubilization was definitely effected. However, losses of tannin and degradation in colour occurred. This suggests that, apart from the expensive equipment employed and the severe processing required, the attempts to make a good commercial product were not altogether successful. In addition, it has been recorded that encouraging results were obtained by treatment of the kino with hot solutions of the above-mentioned reagents in open vessels.

At the outset of the investigations now under review, it was decided to seek the simplest process which would give a practical yield of good quality extract. Whereas previous investigations had aimed at maximum yields by severe processing, in this investigation quality and costs of production were placed before quantity. Hence, attention was given to the consideration of (i) the relationship between temperature of extraction and yield of tannin; (ii) solubilization by simple boiling with cheap reagents; and (iii) the elimination of the more difficultly-soluble bodies by sedimentation, followed

* Institute of Science and Industry, Circular No. 8 (1922).

† Council for Scientific and Industrial Research, Circular No. 9 (1927), and Council for Scientific and Industrial Research, Bulletin No. 32 (1927).

by decantation or filtration. As the result of these studies, a process was evolved and employed on a semi-commercial scale in order to manufacture approximately 2 tons of liquid extract of which over half was of a most encouraging quality. Further reference will be made to this extract later.

3. Laboratory Experiments.

(i) *Relationship between Temperature of Extraction and Yield of tannin.*

(*a*) *Under Analytical Conditions.*—A large bulk sample of finely divided kino-impregnated bark was prepared by using the laboratory bark mill and a 20-mesh screen. From this, a series of smaller samples were taken and extracted in duplicate, according to the procedure given in official methods of tan analysis, at various temperatures ranging from room temperature to 100° C. The infusions thus obtained were examined, by the official method, for total solids, total solubles, insolubles, tans and non-tans. Guided by preliminary extractions at the various temperatures, it was possible to arrange the samples of bark so that all the infusions were approximately at the same concentration, i.e., 4 grams of tannin per litre. Apart from temperature, all other conditions were kept uniform according to a standard procedure. The main results of duplicate tests, together with those of duplicate extractions carried out strictly according to the official method (i.e., 2 litres extraction in four hours), are set out in Table 1. In most cases, for the purpose of comparison, the colours of the infusions at 0.5 per cent. tans were determined by means of the Lovibond Tintometer, and these are recorded in the table. However, a better idea of the effect on colour was obtained by a comparative examination of the hide powder samples subsequent to the non-tan determinations.

Table I.—Marri Kino. Optimum Temperature of Extraction.

Temperature.	Total Solids.	Total Solubles.	Insolubles.	Tans.	Non-tans.	Ratio Tans to Non-tans.	Colour by Tintometer at 0·5 % Tans.		
							Red.	Yellow.	Blue.
°C.	%	%	%	%	%				
Room temperature—									
16 ..	27·2	26·6	0·6	21·2	5·4	4·0	..	n.d.	..
30–40 ..	38·0	35·9	2·1	29·4	6·5	4·5	..	n.d.	..
40–50 ..	41·9	38·3	3·6	31·5	6·8	4·6	5·2	15·0	..
50–60 ..	43·9	38·2	5·7	31·9	6·3	5·1	4·9	20·5	..
60–70 ..	47·4	39·6	7·8	32·8	6·8	4·8	5·6	20	..
70–80 ..	47·7	39·8	7·9	33·3	6·5	5·1	6·3	26·3	..
80–90 ..	47·8	39·7	8·1	33·1	6·6	5·0	6·3	26·3	..
90–100 ..	48·5	39·6	8·9	32·8	6·8	4·8	7·1	27	..
100 ..	48·6	39·7	8·9	32·6	7·1	4·6	7·2	27	..
Official Method (2 litres—4 hours) ..	49·2	40·4	8·8	33·3	7·1	4·7	..	n.d.	..

By reference to Table 1, it will be seen that, at 70–80° C., the maximum extraction of tans was obtained, and that, by increasing the temperature, the extraction of sparingly-soluble matter (officially "insolubles") was increased and the colour intensified. At as low a temperature as 40–50° C., 95 per cent. of the tans were extracted, and the yield of sparingly-soluble matter was considerably less (41 per cent. of the total obtainable), and the

colour was both lighter and brighter. These results suggested that it would be preferable to work at moderate temperatures (say, 60° C.), leaving behind some of the difficultly-soluble material, for the sake of obtaining less turbid liquors and a better colour. Naturally, with less sparingly-soluble matter, the problems of solubilization and clarification would be simplified.

The question as to whether time of contact influenced the extraction as well as temperature was considered by studying the extraction at 70–80° C., using initial periods of contact of $\frac{1}{2}$, 1, and 2 hours, but no appreciable differences in the yields were obtained.

Another point, which was raised during the course of the above experiments, was the possibility of heat causing cold-soluble tannin to become difficultly-soluble. In order to study this aspect, a cold extraction of marri kino was made, and samples of the resulting solution were heated for one hour at various temperatures. The results showed convincingly that the readily-soluble material was entirely stable at these temperatures. It is a fact, however, that, when a clear strong liquor is heated, a precipate is formed, but this readily re-dissolves on dilution and, therefore, is not an "insoluble" in the official meaning of the word. It is suggested that such a reaction results from the reversible coagulation of the soluble bodies at elevated temperatures.

The conclusions that were drawn from the above analytical studies were that extraction at elevated temperatures was entirely unnecessary, and that a satisfactory yield of tans could be obtained at moderate temperatures, such as 60–70° C. At the same time, the sparingly-soluble bodies, known as "insolubles," would not be leached out in such large quantities as they were by previous methods, and, on this account, the clarification and solubilization problems would be simplified. The experiments suggested the following avenues of attack —

(*a*) Leaching at 60–70° C.

(*b*) Clarification by sedimentation, followed by decantation or filtration.

(*c*) Solubilization of the sparingly-soluble tans so that little or no loss would occur during the elimination of bodies still sparingly soluble (and not tannins in the official meaning) by sedimentation and subsequent processes.

Before proceeding with the above, it was decided to confirm the results indicated in Table 1 on a larger laboratory scale. Results obtained under strictly analytical conditions cannot always be repeated on a larger scale. Consequently, the temperature-yield relationship was again studied.

(*b*) *Temperature-yield Relationship under larger Scale Laboratory Conditions.*—For this purpose kino-impregnated bark was disintegrated in the plant bark-chopper-and-grinder employing a $\frac{3}{4}$-in. screen. The resulting material varied in size from fine dust to particles which would not pass through a $\frac{1}{4}$-in. sieve. Consequently, samples drawn from this would very likely have shown considerable variation. In order to minimize this difficulty, the whole sample was sieved into three grades, and each of these thoroughly mixed and weighed. For the actual laboratory samples these grades were taken in their original proportions.

The extractions were carried out according to the customary press-leach system, circulating the "forward" liquor (that which was in contact with the fresh charge of bark) for a period of two hours, so that the whole charge was uniformly at the working temperature, 60° C. It was considered, from

preliminary tests, that all materials extractable at this temperature would then be dispersed, only requiring displacement by weaker washes and finally by water, all at the same temperature, 60° C.

The leaches were carried out in copper beakers, fitted with perforated false bottoms, which were covered with butter muslin. Five such beakers constituted a battery, and the complete leaching of the battery a laboratory test. Each beaker could accommodate 1 lb. of bark together with the necessary amount of liquor (4–4½ parts to 1 part of bark). The ratio of liquor to bark was decided by the strengths of the resulting liquors. It was considered advisable to keep these below 50° barkometer (i.e., sp. gr. = 1.050) in order that large losses would not be sustained during decantation. Yields were determined by weighing and analysing all liquors and washes. Tests were carried out at 60° C., 80° C., and 100° C. In addition, a leach was made at 60° C., and all the resulting strong liquors were placed in tall cylinders in order that sparingly-soluble matter might settle out. In this case only the clear supernatant liquors were considered for the yield. The results served to confirm those obtained under analytical conditions. While the yield of tans was approximately the same for each test, the yield of "insolubles" increased considerably with temperature. The results obtained by clarifying the resulting liquors were of particular interest. They showed that sedimentation and decantation efficiently eliminated the "insolubles," but, without the aid of any solubilizing agent, the accompanying loss in tans was such that the procedure would be impracticable from the commercial point of view.

(ii) *Consideration of Solubilizing Agents.*

When it had been demonstrated that temperature control would greatly contribute towards the production of a useful extract, it was necessary to consider the question of a suitable solubilizing or dispersing agent to render the sparingly-soluble tans cold-soluble so that clarification could be achieved without much loss of tannin. A large number of possible reagents were considered from the following aspects :—

(*a*) the degree of solubilization obtained ;

(*b*) the assistance rendered to decantation and filtration in the elimination of "insolubles" ;

(*c*) the colour of the resulting clarified liquors ;

(*d*) the effect on ultimate yield ; and

(*e*) the cost of reagent.

The following reagents were considered :—Sodium bisulphite, metabisulphite, hydrosulphite ("Hydros"), and sulphite, "Neradol N," "Maxyntan," "Ordavol 2G," "Tannol NNO," "Tannin F.C.," karri extract, "Celltan," and, in addition, the following mixtures :—Sodium bisulphite and "Tannol NNO," sodium bisulphite and "Tannin F.C.," sodium bisulphite and sulphite, sodium sulphite and "Tannol NNO."

Of the above, "Neradol N," "Maxyntan," "Ordavol 2G," karri extract and "Celltan" showed little or no solubilizing properties. The others solubilized to various degrees, the most satisfactory results being obtained from using sodium bisulphite and sodium sulphite (separately). Taking into consideration the above-mentioned aspects, sodium bisulphite (containing over 62 per cent. total SO_2 or 85–90 per cent. estimated as metabisulphite) was determined to be the most useful and convenient material. It was noted in the tests that the quality of the bisulphite considerably influenced sedimentation. With bisulphite of the above-mentioned quality, the "insolubles" settled out as a compact rubbery mass on top of which occurred

a thin layer of granular material. Decantation was very easily and effectively carried out under these circumstances, and, in addition, the rubbery mass promised to be a convenient filtering cake on a larger scale. With poorer quality bisulphite, the "insolubles" did not readily settle, but remained in partial suspension and rendered clarification somewhat difficult. Consequently, losses of tannin in solution occurred as the result of inefficient decantation. The influencing factor was undoubtedly the hydrogen-ion concentration of the resulting mixture of bisulphite and marri extract. Following the satisfactory results obtained with bisulphite as a solubilizer, some laboratory tests were made employing the reagent subsequent to extraction and prior to clarification by sedimentation and decantation.

(iii) *Laboratory Tests employing Sodium Bisulphite as an external Solubilizer.*

For these tests, the extraction was carried out according to the procedure employed in the temperature trials. The strong "forward" liquors were withdrawn after two hours' contact and circulation at 60° C., and were then treated with bisulphite at 100° C. for one hour, allowed to cool slowly, and, while still warm and clear, were poured into cylinders and set aside for a period of two to three days in order to permit the cold-insoluble matter to settle out. It was realized that, on a commercial scale, the treated liquors would cool very slowly, thus affording the bisulphite greater opportunities for dispersing the tannins and, in addition, retarding any sedimentation. This difficulty was overcome to some extent in the laboratory by heating the liquors in flasks immersed in a 10-gallon copper containing boiling water and allowing them to cool with the water in the copper. After cooling, the supernatant liquors were withdrawn by decantation, weighed, and analysed. The weaker washes, occurring at the end of each test, were not treated with bisulphite, but were weighed and analysed in order to be included in the yield determinations. If anything, this latter procedure adversely affected the results, because the "insoluble" content of the washes was proportionately greater than that of the treated liquors.

The quantity of bisulphite used was calculated as a percentage of the total solids contained in the liquor. In order to determine rapidly the total-solids content of a liquor, the barkometer strength at 50° C. was employed. As the result of a number of trials, a fairly definite factor, relating barkometer strength to total-solids content, was determined. At the working temperature, 50° C., all the sparingly-soluble material was still well dispersed, and, therefore, acted as solubles in the barkometer determinations. This temperature was also advantageous in that the delay necessary between the withdrawal of a liquor and its treatment with the solubilizer was reduced to a minimum. The method, at any rate, was sufficiently accurate for the purpose of approximately controlling the quantity of bisulphite used, and promised to be readily applicable on plant scale.

The results obtained from eight laboratory trials are set out in Table 2. There is obviously no definite gradation in these, due, in some measure, to the different conditions occurring from one test to another. Thus no attempt was made to standardize the limits to which each solution cooled, because no such procedure would be practicable on a large scale. The laboratory temperature varied considerably from week to week, and, consequently, the liquors of one run could easily have attained a lower minimum temperature than those of another during sedimentation and also at the time of decantation. These variable conditions naturally influenced the "insoluble" content when "insoluble" meant "not filterable at 20° C." It was, of course,

realized that the large quantities of liquor, obtained on a commercial scale, would not be so susceptible to local daily temperature variations. In addition, the samples of bark used in these tests varied slightly in analysis from test to test (see footnote, Table 2).

TABLE 2.—MARRI KINO-LABORATORY TESTS.

Extraction by press leach at 60°C. and treating liquors externally with bisulphite prior to sedimentation and decantation.

Bisulphite to total Solids.	Net Yield after deducting Reagents added (assuming Non-tans) % O.D. Bark.				Percentage Extraction Obtained.*			
	Total Solubles.	In-solubles.	Tans.	Non-tans.	Total Solubles.	In-solubles.	Tans.	Non-tans.
%	%	%	%	%	%	%	%	%
3 (*a*)	37·6	2·0	30·6	6·9	95	27	95	93
4 (*d*)	35·9	1·9	29·4	6·5	94	23	95·5	88
5 (*b*)	35·3	0·9	28·9	6·4	92·5	9·7	96·0	80
6 (*c*)	36·9	1·6	29·9	7·0	92	25	93	89
7 (*a*)	38·7	1·2	31·4	7·2	98	16	97·5	97
8 (*a*)	36·9	2·2	30·4	6·5	93	30	94·5	88
9 (*c*)	35·4	1·8	29·0	6·4	89	29	90	82
10 (*b*)	33·5	1·1	27·7	5·8	88	12	92	72·5

* *Note.*—Calculated from yield obtained and possible yield as indicated by the analysis of the bark using the official method.

Analysis of bark samples—

	Total Solids.	Total Solubles.	Insolubles.	Tans.	Non-tans.
(*a*)	47·0	39·6	7·4	32·2	7·4
(*b*)	47·4	38·1	9·3	30·1	8·0
(*c*)	46·3	40·0	6·3	32·2	7·8
(*d*)	46·0	38·2	8·4	30·8	7·4

The outstanding results were those in which bisulphite was employed in the proportions of 5 per cent. and 7 per cent. of the total solids present. The former provided a comparatively low yield of "insoluble" matter without any proportional loss in tans. As already indicated, this may have been due, in part, to local temperature conditions. It appeared that 5 per cent. to 7 per cent. of bisulphite could be advantageously used as an external solubilizer, the extra bisulphite affording greater bleaching facilities and, therefore, a brighter coloured product. When used in quantities above this proportion, acidity influenced the yield of tans adversely (see Table 2). The colour imparted to hide substance by the solutions obtained in these tests was highly satisfactory.

The results at this stage indicated a definite procedure for plant tests, viz., leaching at 60° C., and subsequent treatment of all "forward" liquors at 100° C. with bisulphite in the proportion of between 5 per cent. and 7 per cent. of reagent to total solids present.

(iv) *Laboratory Tests employing Sodium Bisulphite as an Internal Solubilizer.*

Before proceeding with plant tests to confirm the previous results, laboratory trials were made adding sodium bisulphite to the leach liquors during extraction and circulation. The procedure adopted for leaching, solubilization, and clarification was similar to that already described. The results showed the effect of the greater solvent power of the bisulphite solution at 60° C. Bodies, which were completely insoluble in water at 60° C., were leached out, thus affording much greater yields not only of tans and non-tans, but also of "insolubles" and red-coloured material. As a

result, clarification was not so readily accomplished, and the colours imparted to hide substance were not at all encouraging. Because of these latter difficulties, this procedure was not further investigated. However, if any work is attempted in the future, it is recommended that this treatment should be given further consideration. Possibly, the use of a minimum quantity of the reagent during leaching and the addition of more reagent preparatory to solubilization at 100° C. may afford more satisfactory results.

(v) *Laboratory Tests with Mixtures of Aluminium Sulphate and Sodium Bisulphite as External Solubilizers.*

Several references have been made in tannin extract literature to the possible use of mixtures of aluminium sulphate and sodium bisulphite for the dispersion of sparingly-soluble tans. According to the theories advanced, free sulphur dioxide is formed *in situ*, thus affording more concerted action of the reagent. In addition, the red-coloured tannins are supposed to form lighter-coloured aluminium salts. The formation of aluminium hydroxide and the introduction of its coagulating properties has been claimed to facilitate clarification. All these claims appeared to promise the solution to the problem of providing a light-coloured and clear marri extract, and the use of these mixtures was accordingly given some attention. The procedure adopted was similar to that when bisulphite was used alone. The results indicated that a considerable loss in tans occurred and that clarification was not effective, resulting in a high yield of insoluble matter. It should be mentioned, however, that the colour imparted to hide substance was very satisfactory, although solutions resulting from the bisulphite treatment gave equally encouraging results.

4. Plant Experiments.

(i) *General Discussion.*

The plant experiments to be described below were not completed before the termination of tannin extract investigations in accordance with a plan to concentrate the staff of the Division of Forests Products in Melbourne.

Owing to the limited time available, the plant experiments now under review were planned not so much to study the question of costs nor to indicate the most desirable technique in any great detail, but rather to confirm or disprove some ideas with reference to solubilization and clarification that had been formulated as the result of laboratory experience. In addition, it was hoped to overcome any difficulties which were not encountered in the laboratory tests, and to indicate the lines of attack for any future work that might be undertaken. The plant tests would also provide some sort of extract which could be used for subsequent decolorization and tanning experiments. Consequently, one short test was carried out before suspending operations entirely, and an account of this is given below.

(ii) *Preparation of Material.*

Kino-impregnated marri bark (9,110 lb.) was disintegrated in a Van Gelder crusher and grinder, using both 1-in. and $\frac{3}{4}$-in. screens. Of this, approximately 6,000 lb. were prepared while the latter screen was in use. The average moisture content was found to be 11 per cent., so that the moisture-free weight of material treated amounted to 8,100 lb. The disintegrated material was elevated to a hopper bin, from which it was discharged into tared bags and weighed. The experience gained during the plant tests showed that the material passing through the $\frac{3}{4}$-in. screen was most readily handled and leached, since a larger percentage of fine kino was obtained, giving rise to a more rapid solution in water.

(iii) *Leaching of Material.*

Each vat was charged with 1,500 lb. of disintegrated material. The charges were sampled during the process by making a grab sample from each 100-lb. lot. The aggregate sample was then quartered down to a convenient size and ground for analysis by the official method. To be more thorough, each of these larger samples should have been graded and each grade separately quartered down. However, since the yield was not the most important factor to be studied during the plant run, this precautionary measure was not taken. It has already been mentioned that it was difficult to take two similar samples of the bark. Hence it was not surprising to find that the six samples obtained differed considerably.

To the 1,500 lb. of bark in each vat, 550 gallons of water or weak wash liquor (during continuous operations) at 60° C. were added. The temperature of the mixture was maintained at 55–60° C. for three hours by circulating the liquor through a tubular water heater. By this time the solution of all material soluble at this temperature was complete and the liquor was withdrawn, at the same time being replaced by weaker washes or water also at 60° C. The strong liquor was passed on to the next charge and the process continued as outlined above. The strong liquor resulting from this second extraction was found to run at about 30° barkometer at 20° C., and was withdrawn to the storage tank for subsequent treatment. The process was continued in the same way (i.e., press leaching at 60° C. with circulation in the forward vat and withdrawal of the strong liquor from each alternate vat) until all six vats had been leached.

It is necessary at this stage to discuss certain mechanical difficulties which were encountered during the leaching process. The ground bark consisted of light porous bark and finely ground denser kino, which readily softened in warm water before dissolving. Consequently, when the leach liquor was run into the vat the charge rapidly separated into two layers. The bark floated and was difficult to wet, and the kino formed a soft impervious layer at the bottom and prevented adequate circulation. It was necessary to break up this layer by means of poles before proceeding with the treatment. The problem appears to be purely mechanical in nature, but it will have to be surmounted before any future work along these lines is attempted. It is possible that the difficulty may be overcome by the simultaneous admission to the extraction vat of both bark and leach liquor through the agency of an intermediate mixing device. The gradual introduction of the bark to the leach liquor contained in the vat and rapid circulation so that the temperature (60° C.) is maintained by means of closed steam coils is also suggested. The admission of a small amount of live steam in advance of the leach liquor might bring about the desired wetting of the bark. A further suggestion is the introduction of a short cold leach preparatory to the admission of hot leaching liquor. Consideration could also be given to the question of finer disintegration of the bark. A mechanical separation of the bark from the gum and the separate treatment of each also presents a possible solution to the problem.

(iv) *Solubilization, Clarification, and Concentration.*

The strong liquors, upon withdrawal, were treated in large copper tanks with sodium bisulphite (containing 62 per cent. total sulphur dioxide or 85–90 per cent. estimated as sodium metabisulphite), using 4 parts to every 100 parts of solids in solution, in the case of the first two cases,* and 7 parts for the remaining four.

* Instead of awaiting the results of analyses, it was possible, from previous laboratory experience, to determine approximately the total solids content from the barkometer reading at 50° C. It was found, however, by subsequent analyses, that the laboratory factor differed from that required in the plant, because of the different bark stock used and consequently a different ratio of tans to non-tans. This matter was remedied, but not before the first two liquors had been treated. Consequently, these only received four parts of bisulphite instead of the five that had been intended.

When the bisulphite had been added to the liquor, the whole was thoroughly mixed and then heated by means of steam coils to 95–100° C. After standing overnight, the liquor was gravitated into a settling vat to permit the solid particles that were not dissolved by the bisulphite (i.e., fine bark, &c.) and those that were still cold-insoluble to settle. This vat was provided with a perforated copper false bottom, which was covered with hessian. The degree of cooling largely influenced the "insoluble" content of the resulting liquor and also the ultimate extract. Since it was midsummer when the experiments were carried out, it was very difficult to have liquors cooled below 27–30° C., whereas 20° C. would have been more desirable. When thoroughly cold (a matter of days, the actual time depending on local conditions), the liquor was slowly pumped from the vat through the false bottom. At this stage, conditions different from those experienced in the laboratory, but not in the least unexpected, were experienced. The large mass of liquor had cooled more slowly than the smaller quantities handled in the laboratory. Consequently, the bisulphite was afforded prolonged opportunities for solubilization. In addition, the same degree of cooling was not attained. These differences were responsible for the presence of only a small amount of insoluble matter at the bottom of the vat. Although this was hardly sufficient to provide a filtering cake, the withdrawn liquors were very clear, suggesting that the "insoluble" bodies had all been retained on the hessian. The clarified liquors obtained during the plant experiments were found to range from 35–38° barkometer.

By the treatment outlined above, there were no losses of soluble substances, but quantities of cold-insoluble red substances were most certainly either eliminated or dispersed. The clear liquors thus obtained were subsequently concentrated to approximately 250° barkometer in a double effect evaporator, run into casks, and stored. The evaporation presented no serious difficulties, but it was necessary to give fairly constant attention in order to effect such a concentration in two stages.

(v) *Consideration of Yields.*

It has already been mentioned that the plant tests were not planned to study the question of yields. However, some data were collected mainly to prove that no losses of tans were incurred during the clarification process. Reference has been made to the difficulties encountered in leaching and the necessity for the breaking up of the cake of kino in order to permit circulation. This naturally encouraged channelling, and it was not surprising, therefore, on emptying the vats to find large lumps of undissolved kino. Consequently, the yields indicated below should be largely improved upon when the leaching difficulty is overcome.

The average analysis of the six samples collected while charging the six vats, expressed on the moisture-free basis, was as follows :—

	%
Total solids	45·9
Total solubles	38·1
Insolubles	7·8
Tans	31·6
Non-tans	6·5
Ratio tans/non-tans	4·9

The total yield, determined by considering the quantities of liquid extracts obtained and their analyses, together with the quantities and

analyses of weak wash liquors that remained at the end of the test, expressed as percentages of the bark, on the moisture-free basis, was as follows :—

	%
Total solids	35·8
Total solubles	34·0
Insolubles	1·8
Tans	26·8
Non-tans	7·2
Ratio tans/non-tans	3·7

This, however, included the 166 lb. of bisulphite, or residual portion thereof, added for the purpose of solubilization. This represented 2·1 per cent. of the bark treated, but some of this was eliminated as sulphur dioxide during the solubilization boil and the evaporation. These losses could not be accurately accounted for, but an assumption that the residual part amounted to 2·0 per cent. of the bark in the yield results was regarded as safe. Again, it was very questionable whether the bisulphite residue could all be accounted for as non-tans. Hence, any definite expression of yield of tans and non-tans could not be made.

It could only be stated that the net yield, exclusive of bisulphite, was :—

	%
Total solids	33·8
Total solubles	32·0
Insolubles	1·8

Since the ratio of tans to non-tans in the bark was 4·9, and since laboratory results had shown that the tans were as readily extracted as the non-tans at 60° C., the extraction or yield ratio could have attained the above figure. By assuming 4·9, the following would have been the approximate yield :—

	%
Tans	26·6
Non-tans	5·4

and, hence, the percentage extraction would have been :—

	%
Total solids	73·6
Total solubles	84·0
Insolubles	23·0
Tans	84·0
Non-tans	83·0

With a solution to the leaching problem, it could confidently be expected that the percentage extraction yield of tans would exceed 90 per cent., and thus attain the standard of the laboratory results.

The yield of total solids and solubles was studied from another aspect. All strong liquors at withdrawal and prior to treatment, as well as the weak washes at the end of the test, were accurately measured and sampled. From these observations, the following yields were determined :—

	Percentage of moisture-free Bark.
Total solids	37·0
Total solubles	32·0
Insolubles	5·0

Hence, thanks to the solubilizer, the elimination of about two-thirds of the "insolubles" was accomplished, by dispersion and sedimentation, without any loss whatever of solubles.

The spent bark still contained a fair amount of soluble tans. This was naturally influenced by the inclusion of large pieces of kino which had caked and which had not been leached on account of the channelling which occurred during the process. Owing to its heterogeneous nature, accurate sampling and analysis of the spent bark were impossible. From the point of view of clarification, the plant tests were most satisfactory. They indicated, however, that a large capacity, in the form of settling vats, would be required on a commercial scale.

(vi) *Consideration of Liquid Extract obtained.*

The following are the analyses of the six liquid extracts obtained :—

TABLE 3.

Serial No.	Total Solids.	Total Solubles.	In-solubles.	Tans.	Non-tans.	Ratio Tans/ Non-tans.	Colour at 0·5 % Tans. By Lovibond Tintometer.	
							Red.	Yellow.
	%	%	%	%	%			
1	46·3	43·5	2·8	34·6	8·9	3·9	11·5	28
2	47·8	45·1	2·7	36·2	8·9	4·2	9·6	25
3	51·1	49·4	1·7	38·7	10·7	3·6	10·3	20
4	49·3	47·4	1·9	37·0	10·4	3·6	10·6	25
5	48·2	46·6	1·6	36·6	10·0	3·7	10·6	25
6	51·0	49·3	1·7	38·6	10·7	3·6	10·6	25

Extracts 1 and 2 were obtained by evaporating liquors which had been treated with 4 per cent. of bisulphite (to total solids present). The other four were treated with 7 per cent. of bisulphite, and a marked improvement in the colour imparted to hide substance by the extract was noticed. The "insolubles" content was also considerably reduced. With reference to this there were probably a number of contributory causes.

(1) Increase in bisulphite.

(2) Owing to a heat wave, the first two liquors did not cool below 30° C. in five days. They were optically clear at this temperature, and were evaporated without further delay. In addition, the major portion of these liquors was decanted and not filtered through the hessian in the settling vat. The later liquors attained the minimum temperature of 26° C. and were all filtered.

(3) A certain amount of deposit from the evaporator tubes found its way into Extracts 1 and 2 before this trouble was noticed.

When the colours imparted to hide substance were considered, it was found that the extracts did not compare very favorably with the head liquors nor with the liquors obtained during laboratory tests, although they were still quite satisfactory. This was not unexpected, partly because of the sustained heating to which plant liquors were necessarily subjected, together with more complete solubilization, but mainly because of the elimination of all free sulphur dioxide during the solubilization boil and throughout the evaporation. That the sulphur dioxide and its bleaching

properties greatly influenced the colour imparted to hide substance was fully appreciated during the laboratory tests. The liquid extracts, on examination, were found to be entirely free from sulphur dioxide and almost free from sodium bisulphite.

Attempts to improve the colour of the extract prepared by using 7 per cent. bisulphite as solubilizer were subsequently made in the laboratory. A large number of reagents were tested, and these included organic and inorganic materials which have been mentioned in the literature as being efficient decolorizing agents. Several syntans were included in the tests, but without success. Blending marri extract with karri extract did not yield any fruitful results. The most satisfactory results were obtained by the addition of sodium bisulphite, hydrosulphite, or metabisulphite, or free sulphur dioxide to the extract. These were added in definite proportions related to the total solids content and the extract was heated to 50° C. Sodium bisulphite and sulphur dioxide were found to be the most satisfactory, mainly on the score of cost. The addition of an extra 1 per cent. showed marked improvement in colour, although it was considered that 3 per cent. gave the most satisfactory results. The above percentages are based on the total solids present.

It was concluded that the most satisfactory extract resulted from solubilization with 7 per cent. bisulphite and subsequent additions to the extract of 3 per cent. bisulphite or 1–2 per cent. of free sulphur dioxide. The total added material would therefore not exceed 10 per cent. of the total solids leached from the bark, or about 4 per cent. of the total bark extracted. The conditions in the case of a solid extract would naturally be somewhat different.

Several small samples of extract treated during these decolorization tests were prepared for tanning trials, but, to date, no facilities have been available for making these tests. The bulk of the extract obtained in the plant was not treated in this way, but was sold to a local tanner, who has reported very favorably on its behaviour.

5. Conclusions.

As the result of laboratory and plant scale tests, it has been shown that a satisfactory extract can be made from kino-impregnated marri bark. It would appear to be preferable to forsake 100 per cent. yield of tans in order to produce a brighter coloured and clearer extract. Leaching at 60° C. with water or weak washes and subsequent treatment at 100° C. with sodium bisulphite, amounting to about 7 per cent. of the total solids, followed by settling, has been found to yield clear, bright-coloured liquors which can be concentrated to a clear extract with a satistactory colour. The colour of the latter may be further improved by the addition of small quantities of sodium bisulphite or of sulphur dioxide.

Certain difficulties have still to be overcome, the main one occurring in the leaching process, but this appears to be simply of a mechanical nature.

Foot and Root Rots of Wheat in Australia.

Fusarium culmorum (W.G.Sm.) Sacc. as a Causal Organism.

By W. L. Geach, B.Sc.*

Summary.

1. Numerous cultures of Fusaria were isolated from foot-rooted wheats obtained from various wheat-growing areas in the Commonwealth. Many of the Fusaria were found to belong to the group "Discolor."

2. Of the Fusaria of the "Discolor" group, *F. culmorum* (W. G. Sm.) Sacc. was identified. Two isolations of *F. culmorum* were selected and compared with a culture of this organism from Saskatchewan, and close agreement regarding the measurement of sporodochial conidia was obtained.

3. A number of wheat varieties were inoculated with transfers from these cultures, and the pathogenicity of the organisms was established, both in the glasshouse and in the field, after preliminary trials on plants grown in artificial media in large glass tubes.

4. *F. culmorum* produced typical foot-rot symptoms, including the condition known as white heads, usually attributed to *O. graminis*.

5. Of the eighteen varieties of wheat so far tested, none showed immunity to the isolations of *F. culmorum* used in the experiments reported in this paper.

1. Introduction.

The group of wheat diseases to which the names foot-rot, root-rot, take-all, white heads, and others are variously applied, is the most economically important in Australia. Brittlebank (4) of Victoria, writing on this subject, states, "Of all the fungus diseases affecting wheat, 'take-all' is the most destructive, and the actual loss caused by it is far greater than by any other single disease, rust included, or perhaps by a combination of all known fungus diseases affecting wheat in Australia." In a recent publication, Hynes (8) reports that foot-rot is one of the most serious diseases in New South Wales, and he gives several instances of 30%–50% losses. Carne (5) also reports 30%–50% reduction in estimated yield in some districts in Western Australia, and states that 10% loss is common. In an anonymous article in the *Journal of the South Australian Department of Agriculture* (1) written in 1906, a year when the disease was unusually severe, it is stated:—"In some cases the prospective yield of a whole district is reported to have diminished 30%." Mackinnon (10) considers the loss throughout the Commonwealth caused by take-all to average 7% per annum. The writer estimates a crop reduction of 30%–75% in some fields visited by him during the past season. Estimating the average annual yield reduction at the very conservative figure of $2\frac{1}{2}$% of the total wheat crop, the loss for Australia as a whole based on a five-year average of production and prices amounts to about £750,000 per annum. It is probably much greater.

According to the literature, the organisms associated with the disease complex in Australia are *Ophiobolus graminis*, Sacc., *Wojnowicia graminis* (McAlp.) Sacc., *Helminthosporium sativum*, P.K.B., *Rhizoctonia* sp., *Brachysporium* sp., and occasionally mention is made of Fusaria. In 1921, Hamblin (6) working on *Helminthosporium*, noted the frequency of occurrence of Fusaria in his isolations, but

* Assistant Plant Pathologist, Division of Plant Industry, C.S.I.R.

no mention was made of attempts to determine their importance. In 1924, Hynes (7) recorded the occurrence of *Gibberella saubinetii* (Mont.) Sacc. in New South Wales when he identified perithecia on oat-stubble as those belonging to that organism. On plating out some of the perithecia, he obtained cultures of a Fusarium which agreed with the description given for the conidial stage of *G. saubinetii,* but he was, however, unable to obtain the perfect stage in pure culture. He showed that this organism was pathogenic on wheat and other cereals, producing foot-rot symptoms.

Numerous cultures of Fusaria of the "Discolor" type* have been isolated by the writer from diseased wheat plants obtained from many districts in the principal wheat-growing areas of the Commonwealth. Specimens from the Wimmera district, particularly, have yielded almost without exception cultures of Fusaria macroscopically similar to isolations identified as *F. culmorum,* although this organism has not been reported hitherto as causing foot-rot of wheat in Australia. In 1896, McAlpine (9) described *F. culmorum* (McAlp.) as occurring in salmon-coloured patches on wheat, particularly at the nodes and on the ears, but he did not give an opinion regarding its pathogenicity. According to Simmonds (11), *F. culmorum* causes seedling-blight and foot-rot of oats in Canada, and Bennett (2) reports it as a cause of foot-rot of wheat in Great Britain.

The object of this paper is to record the occurrence of *F. culmorum* (W. G. Sm.) Sacc. in Australia as a causal organism of foot-rot of wheat, to show that it is pathogenic not only in the glass-house, but also under field conditions, and to indicate its importance in this country. Studies of the effect of environmental factors on the incidence of the disease, and testing of numerous wheat varieties for relative resistance, are now in progress.

2. Source of Material.

The State Departments of Agriculture of New South Wales, Victoria, South Australia, and Western Australia very courteously co-operated by sending in specimens of foot-rotted wheats to the Division of Plant Industry at Canberra during the past two years. Those from the 1931 crop were obtained from seven districts in New South Wales, seventeen in South Australia, ten in Victoria, and six in Western Australia. Several hundred diseased plants were also collected by the writer and others from the New South Wales wheat areas of Wagga, Coolamon, Junee, Temora, Wyalong, Young, and Cowra.

3. Isolation and Identification of Organism.

Isolations were made from lesions on the roots and on the basal portions of the stems. The selected portions were washed in water to rid them of loose earth, immersed in 70% alcohol for 1½ minutes, washed in sterile water, transferred to potato-dextrose agar in petri dishes, and a drop of 5% lactic acid added around each piece of material to prevent growth of bacteria. After a few days, portions of the developing fungi were transferred to potato-dextrose agar in tubes.

Several hundreds of "Discolor" Fusarium isolations were made, and a number of them were studied culturally and microsopically.

* In this paper the term "Discolor" is applied to those Fusaria which, when grown on potato-dextrose agar, produce bordeaux, rose, honey, and white coloured hyphae, accompanied by the production of bordeaux pigment in the agar.

Although the cultures appeared to be of similar appearance macroscopically, certain differences were noticed when they were more closely examined, and therefore, with the object of arriving at some satisfactory and rapid way of grouping them by comparison of their gross characters, they were grown on a variety of media, including potato-dextrose, plain potato, oat, wheat, salts glycerin, starch asparagin agar, melilotus stems and potato plugs. Such attempts, however, gave unsatisfactory results, since perplexing variations occurred under similar environmental conditions.

No perithecia were observed in any of the cultures, although many attempts were made to induce their formation.

Two of the isolations were selected for the studies reported in this paper. One of them was from the wheat variety "Purple Straw" from Longerenong, Victoria, indexed in the writer's collection as F. 23, and the other from the variety "Federation" also from the Wimmera district, indexed as F. 30. Incidentally, it may be mentioned that a species of *Helminthosporium* (probably *sativum*) was also isolated from the material from which F. 23 was obtained.

A culture of *F. culmorum* from Saskatchewan, Canada, and the two isolations F. 23 and F.30 were grown at the same time and under the same environmental conditions on sterilized melilotus stems, and measurements of the sporodochial conidia of all three cultures were made. In the following table, it will be seen that the conidial measurements of the Australian isolations showed close agreement with the Saskatchewan culture.

Table I.—Measurements of Sporodochial Conidia.

Number of Septa.	*F. culmorum* (Saskatchewan).	Percentage of each Type.	F.30.	Percentage of each Type.	F.23.	Percentage of each Type.
5	34·5 x 5·2 μ	26	33·9 x 4·79 μ	33	34·07 x 4·73 μ	53
4	32 x 4·7 μ	49	30 x 4·9 μ	34	30·33 x 4·3 μ	24
3	28·8 x 4·7 μ	23	26·7 x 4·34 μ	32	25·22 x 4·4 μ	23
2	..	..	25 x 5 μ	1		

Subcultures of F.23 and F.30 were finally submitted to Dr. H. W. Wollenweber of the Biologische Reichsanstalt für Land- und Fortswirtschaft, Berlin-Dahlem, Germany, who identified them as *F. culmorum* (W. G. Sm.) Sacc.

4. Inoculation Experiments.

(i) *Preparation of Inoculum and Grain.*—Wheat grain with about an equal volume of water, was sterilized in Erlenmeyer flasks at 15 lbs. pressure for 45 minutes on two successive days. On this medium, the isolations F. 23 and F. 30 were grown in quantities sufficient for soil inoculation. Kernels of the wheat varieties to be sown were soaked over-night in water, and surface-sterilized by steeping for three minutes in a 50% alcohol solution containing mercuric chloride (0.2%), as recommended by Bennett (2).

(ii) *Inoculation of Plants in Pure Culture.*—As a preliminary to glass house and field trials, wheat plants were grown in large glass tubes

18 in. x. 2 in. on a medium made by adding 2% agar to the pH 6.2 culture solution of Brenchley, Maskell and Warrington (3). Five surface-sterilized kernels were placed on the surface of the agar in the tubes and a small quantity of the inoculum added.

Although this method involved the growth of the host plant under artificial conditions, it served a useful purpose, since all organisms other than the selected one were excluded, the condition of the root system of the plants could be observed, and it was possible conveniently and quickly to obtain some idea of the relative virulence of the isolations tested.

The wheat varieties grown in the tubes with the inocula F. 23 and F. 30 respectively, were rapidly attacked and killed. A number of other isolations of similar macroscopic appearance were also tested against wheat plants in this manner with essentially similar results. Other plants in tubes in which common saprophytic fungi such as *Macrosporium, Mucor* or *Penicillium* were allowed to grow, showed no foot-rot symptoms.

(iii) *Glasshouse Experiments.*—The soil used in these experiments consisted of five parts of river sand and three parts of white sand, thoroughly mixed and afterwards sterilized for four hours by steam at atmospheric pressure. An equal amount of soil was put into each container, also previously sterilized. The grains, surface sterilized by the method already indicated, were planted at a depth of $\frac{3}{4}$ in., and a piece of inoculum about the size of a wheat grain placed in contact with each before covering with soil. Fourteen varieties of wheat were used in these experiments. Ten grains of each variety were inoculated with F. 23, and ten of each with F. 30. Twenty grains of each variety served as checks for both sets of experiments.

A weighed quantity of a sterilized gravel mulch was spread over the soil to conserve moisture. Water was weighed in to the extent of the field-carrying capacity of the soil used, and the loss by evaporation made up once a week.

Results of the experiment, after six weeks' growth, are given in Table 2. It will be observed from the figures given in the control column that the average germination capacity of the grain was good, except in the case of the varieties "Major" and "Ford" Comparing the control figures with those in the column headed F. 23, it will be seen that this organism was responsible for a relatively large amount of disease which was partly manifested as seedling-blight and partly as blighting before emergence. The same symptoms were also observed on the plants inoculated with F. 30 but the percentage of infection, as indicated in the table, was lower than that produced by F. 23.

In a number of cases where no plants appeared after germination was general, the grain was dug out and examined, and in many instances it was seen that although germination had begun, the fungus had attacked and killed the developing seedlings before their appearance above the soil. Some plants grew to a height of one to two inches, then yellowed and died back. The region around the foot of such plants was rotted and of a deep brown colour. Isolations from the dead and dying plants gave pure cultures of a Fusarium indistinguishable in general appearance from F. 23 and F. 30. No foot-rot was observed in the controls.

Other wheat varieties were also tried and the isolations were found to be pathogenic on them.

TABLE 2.

Wheat Variety.	Inoculum F.23. 10 Grains Sown.		Inoculum F.30. 10 Grains Sown.		Controls. 20 Grains Sown.		Average Germination Capacity Per 10 Seeds.
	Healthy Plants Remaining at end of Experiment.	Plants diseased, Dead, or did not appear above Ground.	Healthy Plants Remaining at end of Experiment.	Plants diseased, Dead, or did not appear above Ground.	Healthy Plants Remaining at end of Experiment.	Plants diseased, Dead, or did not appear above Ground.	
Baroota Wonder ..	6	4	8	2	19	1	9·5
Sunset	1	9	3	7	18	2	9
Ford	0	10	4	6	11	9	5·5
Bunyip	0	10	6	4	19	1	9·5
Currawa ..	1	9	7	3	19	1	9·5
Nabawa ..	0	10	1	9	17	3	8·5
Canimbla ..	0	10	4	6	17	3	8·5
Sands	4	6	7	3	19	1	9·5
Major	0	10	6	4	11	9	5·5
Dunmore ..	0	10	8	2	19	1	9·5
Geeralying ..	2	8	5	5	17	3	8·5
Hard Federation ..	2	8	7	3	16	4	8
Joffre	2	8	5	5	19	1	9·5
Caliph	2	8	8	2	18	2	9

5. Field Trials.

Trials were also made in field plots. The soil was inoculated and the grain sown on July 1st, September 9th and October 8th. The first two sowings were in rectangular areas subdivided into plots measuring 3 ft. x 2 ft. Six plants of each of four varieties were sown in each plot, of which there were twenty-one. Three of the plots were picked at random for controls. The last sowing was arranged differently, the grain being sown in six rows with approximately 200 grains of each variety per row. Observations were made from time to time, and as the plants approached maturity, striking differences were noticed between many of the inoculated plants and the controls. The majority of the control plants were moderately well developed, and of normal appearance, whereas the plants growing in the artificially inoculated soil were in many cases severely stunted and almost rotted through at the base of their culms. A number of plants showed the typical white head symptom, usually attributed to *O. graminis* (Plate 2). On examination, the ears were found to contain either shrivelled grain, or none at all.

All diseased plants from the first plot sown, and a number from the other plots were taken to the laboratory and material selected for plating. In nearly every case, cultures were obtained of a Fusarium of the same macroscopical appearance as F. 23 and F. 30. The controls were also carefully examined, but very few plants showed foot-rot lesions. Pieces of material showing these lesions were plated and cultures were obtained of Fusarium and other organisms. The

occurrence of foot-rot in the check plants was expected, as the soil was known to harbour foot-rot organisms. Observations on the reaction of the varieties to the inocula F. 23 and F. 30 are given in Table 3.

TABLE 3.

Inoculum F.30.		Inoculum F.23.	
Wheat Variety.	Observations.	Wheat Variety.	Observations.
Baldmin ..	Medium to severe infection	Ford ..	Medium to severe infection
Bena ..	Slight infection	Florence ..	Medium to severe infection
Bobin ..	Many plants severely foot-rotted	Firbank ..	Slight to very severe infection
Moira ..	Slight to severe infection	Cleveland ..	Slight infection

It is concluded from the results of the field trials that the incidence of foot-rot was due principally to *F. culmorum* with which the soil was inoculated at the time of sowing.

6. Acknowledgment.

The writer desires to express his appreciation of the valuable assistance given in the preparation of this paper by Dr. H. R. Angell, Senior Plant Pathologist, Division of Plant Industry, C.S.I.R., Canberra.

7. Literature Cited.

1. Anonymous.—Takeall in wheat. *Jour. Dept. Agr., S. Aust.* **10**: 280-283, 1906.

2. Bennett, F. T.—Two species of *Fusarium* as parasites of cereals. *Ann. App. Biol.* **15**: 213-244, 1928.

3. Brenchley, W. E., Maskell, E. J., and Warrington, K.—The inter-relation between silicon and other elements in plant nutrition. *Ann. App. Biol.* **14**: 45-82, 1927.

4. Brittlebank, C. C.—Green manurial crops and "take all" *Ophiobolus graminis* (Sacc.). *Jour. Dept. Agr., Vic.*, **17**: 171-174, 1919.

5. Carne, W. M.—Root-rot and foot-rot of wheat. (*Wojnowicia graminis* and *Helminthosporium sativum.*) Agr. Dept., W. Australia. Leaflet No. 228, 6 p.

6. Hamblin, C. O.—Foot-rot of wheat caused by the fungus *Helminthosporium. Agr. Gaz., N. S. Wales*, **33**: 13-19, 1922.

7. Hynes, H. J.—On the occurrence in New South Wales of *Gibberella saubinetii*, the organism causing scab of wheat and other cereals. *Jour. and Proc. Royal Soc. N. S. Wales* **57**: 337-348, 1924.

8. Hynes, H. J.—Foot-rot of cereals. Observations on the disease in New South Wales. *Agr. Gaz. N. S. Wales* **43**: 107-115, 1932.

9. McAlpine, D.—Australian fungi. *Agr. Gaz. N. S. Wales* **7**: 299-306, 1896.

10. Mackinnon, E.—Crop losses. *Science and Industry* (Australia) **2**: 398-407, 1920.

11. Simmonds, P. M.—Seedling blight and foot-rot of oats caused by *Fusarium culmorum.* Dept. Agr. Bull. Dom. Exp. Farms, Canada, 105 (N.S.): 1-43, 1928.

The Nomenclature of Australian Soils.

By J. A. Prescott, M.Sc., and J. K. Taylor,† M.Sc., B.A.*

During the course of soil surveys carried out by the Division of Soil Research during the past three or four years, it has been customary to denote the soil types encountered by means of numbers and letters pending a more general knowledge of the distribution and relative importance of the various soil types recognized. The basis for the definition of any given type has been the soil profile, usually to a depth of 6 feet or to parent rock, soils having profiles uniformly similar in every respect belonging to the same type, while minor variations within the profile could be treated as phases of the type. With increasing experience, it is now becoming possible to give the types recognized specific names, and the binomial system of the United States Department of Agriculture, which has recently been discussed by Lee,(1) has proved to be the most convenient for this purpose. The authors have also been in correspondence with Dr. C. F. Marbut, of Washington, and Professor C. F. Shaw, of California, on the subject. In this system, each soil type receives a "series" name, a geographical term defined by the locality where the type was first recognized or where the soil is most typically developed, and a class name defining the texture of the surface soil, this texture being determined both from field and laboratory examination of the type. No attempt has been made as yet to place closely related soils into "families" or similar associations, but generally speaking each soil type can be readily placed into one of the major soil groups.

Although the word "series" is used for the preliminary grouping of soils having a common profile except for the texture of the surface soil, many soil series contain only one or two soil types, and the use of the word is somewhat unfortunate in that it tends to confuse newcomers to the subject, and obscures the fact that soils belonging to several different series may be just as closely related as soils belonging to the same series. This does not, however, detract from the fundamental soundness of the system.

An examination of the soils so far defined on a profile basis in Australia reveals the fact that each of these recognized soil types conforms either to a soil type as defined by the United States of America system, to a phase of a soil type, or occasionally to a new type within the same family. It has, therefore, proved possible to give names to most of the types already recognized, and the series names have been registered with Professor C. F. Shaw, of the University of California, in his capacity as registrar for the International Society of Soil Science. The following table gives a list of names of the types now recognized, together with references to their published description. In a few cases, recognized types are not sufficiently important to justify naming at the present stage.

* Chief, Division of Soil Research, C.S.I.R.; also Professor of Agricultural Chemistry, Waite Agricultural Research Institute, University of Adelaide.

† Division of Soil Research, C.S.I.R.

TABLE 1.—DETAILS OF THE NAMES OF RECOGNIZED SOIL TYPES WITH REFERENCES TO DESCRIPTIONS AND OBSERVED OCCURRENCE.

Original Type No.	Soil Type Names.	Phases.	Observed Occurrence.	References.
1	Murray sand	.. ..	Renmark (S.A.) ..	(2), (3)
			Mildura (Vic.)	(5)
			Swan Hill (Vic.)	
2	Mobilong clay ..	(*a*) Deep (normal)	Lower Murray River	(4)
2A		(*b*) Medium	Swamps (S.A.)	
2B		(*c*) Shallow		
3	Bookmark sandy loam ..	.. ..	Renmark (S.A.) ..	(2), (5)
3A	Bookmark clay loam ..			
5	Ral Ral sandy loam ..	.. ..	Renmark (S.A.) ..	(2), (5)
5A	Ral Ral clay loam			
5B	Ral Ral loam			
6	Renmark loam ..	.. ..	Renmark (S.A.) ..	(2), (5)
6A	Renmark clay loam			
6B	Renmark sandy loam			
7	Woorinen loam ..	.. ..	Swan Hill (Vic.) ..	(3)
9	Beverford clay loam ..	.. ..	Swan Hill (Vic.) ..	(3)
10	Swan Hill clay ..	.. ..	Swan Hill (Vic.) ..	(3)
..	Millicent clay ..	(*a*) Shallow ..	South-Eastern Dis-	(6)
		(*b*) Deep (normal)	trict (S.A.)	
..	Meadows sand ..	.. ..	Hd. of Kuitpo (S.A.)	(7)
	Meadows fine sand			
	Meadows clay loam			
..	Kuitpo gravelly sandy loam	.. ..	Hd. of Kuitpo (S.A.)	(7)
..	Myponga sand ..	.. ..	Hd. of Kuitpo (S.A.)	(7)
..	Burbook sandy loam ..	(*a*) Normal ..	Hd. of Kuitpo (S.A.)	(7)
		(*b*) Shallow, stony		
..	Currie calcareous sand ..	.. ..	King Island ..	(8)
..	Yambacoona sand ..	.. ..	King Island ..	(8)
..	Nugara sandy loam ..	.. ..	King Island ..	(8)
..	Pegarah fine sandy loam	.. ..	King Island ..	(8)
..	Naracoopa sand ..	.. ..	King Island ..	(8)
..	Lappa sand	.. ..	King Island ..	(8)
..	Taroona sand ..	.. ..	King Island ..	(8)
..	Camp Creek sandy loam	.. ..	King Island ..	(8)

References.

(1) L. L. Lee. (*a*) Imp. Bur. Soil Sci. Tech. Com. No. 6. 1930.

(*b*) *Jour. South Eastern Agric. Coll. Wye.* **28**: 65. 1931.

(2) J. K. Taylor and H. N. England: Coun. Sci. Ind. Res., Aust. Bull. No. 42. 1929.

(3) J. K. Taylor and F. Penman: Coun. Sci. Ind. Res., Aust. Bull. No. 45. 1930.

(4) J. K. Taylor and H. G. Poole: Coun. Sci. Ind. Res., Aust. Bull. No. 51. 1931.

(5) T. J. Marshall and P. D. Hooper: Coun. Sci. Ind. Res., Aust. Bull. No. 56, 1932.

(6) R. Hill, C. S. Piper, and J. K. Taylor: Report to Lands Department, S. Aust.

(7) J. K. Taylor and J. O'Donnell: *Proc. Roy. Soc. S. Aust.* 1932 (in press).

(8) C. G. Stephens and J. S. Hosking: Coun. Sci. Ind. Res., Aust. Bull. (in preparation).

PLATE 1.

(The Root System of the Sultana. See page 88.)

Top left.—A profile at a distance of 2 ft. 6 in. from the butt of a vine.

Top right.—Portion of a main root of a Zante Currant vine. The ascending tendency of the laterals and the effect of injury by the plough are shown.

Bottom left.—The butt of a Sultana vine eight years old.

Bottom right.—The root system of a Zante Currant vine during the course of excavation, showing horizontal spread of roots. The root system of the Currant is very similar to that of the Sultana.

C.4013.

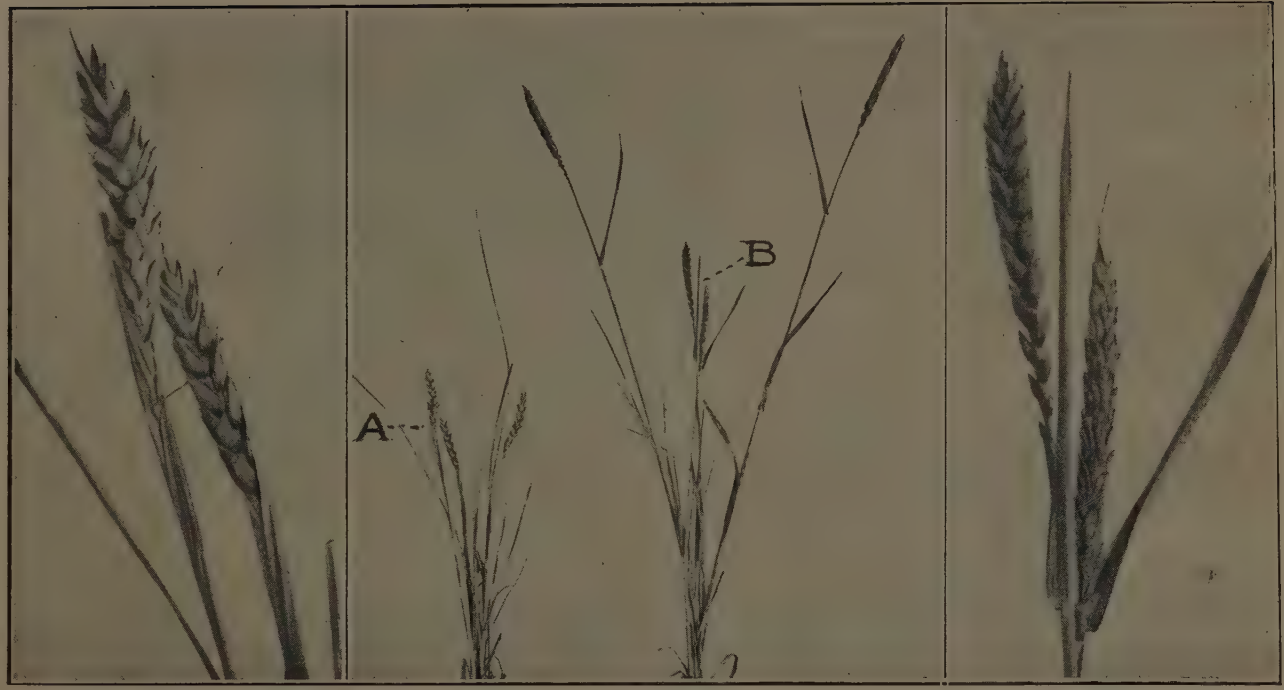


FIG. 2. FIG. 1. FIG. 3.

Comparison of healthy and foot-rotted plants of "Baldmin" wheat.

FIG. 1 (A).—Plant affected by *Fusarium culmorum* (F23), showing stunting and white head symptoms.
(B).—Healthy plant of same variety.

FIG. 2.—Ears of diseased plant. A enlarged to show white heads (nearly natural size).

FIG. 3.—Ears of normal plant B.

NOTES.

Co-operative Investigations into the Problems of the Cattle Industry of North Australia.

As yet, little information has been published regarding the co-operative investigations that have recently been initiated into the various problems of the cattle industry of North Australia. However, now that active work on these problems is about to commence, a brief account of the whole scheme of investigation and of the considerations that led up to its formulation may be of interest.

The proposal began to take shape some years ago when the Empire Marketing Board offered to contribute towards the establishment of a Tropical Research Station in Australia, on a £1 for £1 basis and up to a maximum of £25,000 for capital and £5,000 per annum for maintenance over a period of five years. This offer was made shortly before the holding of the 1927 Imperial Agricultural Research Conference, which, among other things, supported in general the establishment of a "chain" of tropical research stations throughout the Empire.

The scheme was given very careful consideration by the Council (for Scientific and Industrial Research), but as time went on it became increasingly evident that Australian conditions and problems did not warrant quite the kind of station the above-mentioned Conference had in mind. For instance, although quite a large proportion of Australia lies within the Tropics, yet the greater part of that area is comparatively dry and does not possess the luxuriant vegetation of normal tropical regions. Moreover, it became evident that the problems of North Australia were problems connected with the production of animals rather than the production of plant products. Finally, many of the authorities consulted came to the conclusion that it would be better for the Council to establish sub-stations in Queensland to undertake investigations on different tropical problems and to arrange for the efforts of these sub-stations to be controlled by the Council through its various Divisions, rather than to establish a new and independent large station which would after all be concerned with the same branches of science as those already covered by the Divisions.

As a result of these and other considerations, the proposal to establish a Tropical Research Station was modified in the direction of concentration on the animal side as distinct from the plant side. Finally, after numerous discussions between the Empire Marketing Board, the Government and other interested organizations in Queensland, the following scheme of co-operation was agreed upon by all parties.

Co-operating Bodies.—In the first place, Queensland cattle-owners have voluntarily requested their State Government to levy on them to the extent of 1s. per 100 head of cattle they possess. This levy is being made under "The Diseases in Stock and Brands Act, Amendment Act 1931", and all cattle owners possessing less than 100 head are exempt. In this way, approximately £2,000 per annum is being made available for the work.

In addition, the Queensland Department of Agriculture and Stock is making available the sum of £1,000 per annum, which amount it was previously expending at Townsville. It is also making available its laboratory at Townsville. The necessary alterations to this building

are now being made, but they are expected to be complete within a month or so. Finally, the Council of Agriculture in Queensland is also making a contribution of £300 per annum for five years.

All the above contributions are being matched on a £1 for £1 basis by a grant from the Empire Marketing Board, which grant itself is for a period of five years and up to a maximum of £5,000 per annum.

Staff.—The initial staff of the laboratory will consist of A. W. Turner, D.Sc., D.V.Sc., as Officer-in-charge, John Legg, D.V.Sc., R. B. Kelley, B.V.Sc. (Field Officer), and A. D. Campbell, B.V.Sc., Assistant Bacteriologist. In addition a laboratory assistant and a station foreman will be appointed. Dr. Turner left for Townsville in January. Prior to that, he was located at the Veterinary Research Institute, Parkville, where among other things he was responsible for the carrying out of the Division of Animal Health's investigations into black disease of sheep. With the exception of Dr. Legg, the other members of the staff followed Dr. Turner a little later. Dr. Legg has been seconded to the Council from the Queensland Department of Agriculture and Stock, and is now in South Africa inquiring into developments that have taken place there and in particular at the Onderstepoort Veterinary Research Station, regarding ticks and tick-borne diseases.

Programme of Investigations.—The problems which have been included in the initial programme are as follows:—

(i) Ticks and tick fever; (ii) pleuro-pneumonia of cattle; (iii) tuberculosis of cattle; (iv) peg leg disease; (v) walkabout disease in horses; (vi) black leg; (vii) onchocerciasis (worm nodules); and (viii) internal parasites.

In the first year or so, the main problems studied will be (i) to (iv) above, but in periods of low incidence or as the occasion offers, due attention will be given to the other problems mentioned.

(*a*) *Tick and Tick Fever.*—Prior to his visit to South Africa, Dr. Legg had already carried out a considerable amount of work on this problem. On his return, it is proposed that he will make a further study of the tick and those blood parasites conveyed by it in Northern Australia. It is obvious that a knowledge of these parasities is necessary in order that satisfactory immunising agents may be prepared. In addition to this phase of the problem, it is proposed to study what appears to be a natural resistance of certain strains of cattle to ticks, and also certain natural conditions—particularly as existing on one or two stations in Western Australia—which for some hitherto undetermined reason appear to be natural deterrents of the tick.

(*b*) *Contagious Pleuro-pneumonia of Cattle.*—It is proposed to make a further study of diagnostic methods and their practical value, and the degree of immunity induced by a natural infection from which recovery has apparently taken place, in order to determine whether infection ever does clear up and re-infection occur. Exhaustive tests regarding the efficacy of vaccines are considered to be urgently necessary, and if of real value the possibility of discovering more satisfactory means of preparation of such vaccines would be studied.

(*c*) *Tuberculosis in Cattle.*—The present theories as to the means of spread of contagion do not seem adequate to explain the high incidence found on certain tropical properties. Investigations from this point of view are therefore proposed both in the laboratory and in

the field. These will be supplemented by tests with vaccine particularly that known as B.C.G. and later on it is hoped with the Spahlinger vaccine.

(*d*) *Peg-leg Disease.*—The evidence available points to this disease, which affects cattle, being due to a nutritional disorder and possibly a lack of phosphorus. To some extent, however, the evidence is conflicting. It is therefore proposed to study the symptoms, pathological changes, &c., in order that a practical means of preventing the appearance of the condition may be developed.

(*e*) *Walkabout Disease and Poison Plants in General.*—Walkabout disease of horses causes considerable inconvenience to station activities in certain parts of Queensland. The condition as it occurs in Western Australia has been investigated by the Council (see Bulletin No. 36), and as a result, consumption of whitewood by horses is considered to be the cause. In some parts of Queensland, however, the results of this work are evidently not applicable in their entirety. It is accordingly proposed to carry out some investigations at Townsville as occasion offers.

In addition to whitewood, there are various other poisonous plants affecting the cattle industry of the north; and as comparatively little work has been carried out on the matter, it is proposed to investigate various suspected plants.

(*f*) *Black-leg.*—This disease is of no little importance, particularly in Central and Southern Queensland, amongst stud and dairy calves. A solution of the problem would thus be of considerable value to the dairy industry of Queensland.

(*g*) *Onchocerciasis* (*Worm Nodules in Cattle*).—This disease is one of the most severe taxes on the export beef trade that Australia has to contend with. A considerable amount of investigation has already been devoted to the problem by Australian workers, but the assumed insect vector has not yet been discovered.

(*h*) *Advisory Committee.*—It is proposed to set up a small Advisory Committee representative of all the co-operating organizations to advise generally in connexion with the above work which is to be carried out at Townsville as the main centre.

Investigations on the Storage and Transport of Meat.

In the previous issue, a short note dealing with the Council's recently established section of food preservation and transport was given. The section's programme of work on meat and meat products is to be put in hand almost at once, largely as the result of facilities made available by the Queensland Meat Industry Board. The following is a brief account of the proposed investigations and their objects.

The laboratory where this work will be undertaken is being established at the Brisbane Abattoir, Cannon Hill, Brisbane, and will probably be complete in July next. The buildings and equipment will be erected at the expense of the Meat Industry Board, while the Council (for Scientific and Industrial Research) will supply and maintain the necessary research workers. Being linked so closely with a modern meat works erected to deal with the export as well as the local meat rquirements, it is believed that adequate facilities will be available to study any pressing problem connected with the treatment side of the meat industry, and that the usual time lag between the successful solutions of these problems and their application to industry will be greatly reduced.

The laboratories will consist of two rooms fitted for bacteriological, chemical, and physical investigations, and several cold chambers specially fitted to secure strict control of temperature, relative humidity, and the composition and rate of movement of the enclosed atmosphere.

The problems initially to be studied may be summarized as follows:—

Chilled Beef.—Unless Australia is able to supply consumers overseas with beef in a condition closely resembling the fresh article, it is probable that export will continue to diminish in volume; at present the chief outlets for frozen beef in Great Britain are by contracts with military, naval, and poor law authorities, very little passing into general public consumption. Moreover, it is unlikely that the export of packaged quick-frozen cuts of beef will be commercially possible, at least for many years to come. Investigations designed to secure the export in the chilled condition of a considerable proportion of Australian beef would, therefore, seem to be essential if Australia is not to withdraw from the beef exporting trade.

The investigations will be concerned initially with the nature and extent of the deterioration of the fat and flesh of beef held at temperatures approximating to 30 deg. F. for periods up to 60 days in duration. Because microbial attack is likely to be the most serious factor in deterioration, investigations will also be undertaken to determine the best methods of handling and treating sides of beef prior to chilling. Concurrent with these studies, others will be undertaken to determine the optimum temperature, relative humidity, rate of air movement, and composition of the atmosphere necessary during both the initial cooling of the sides and the period of holding at "chill" temperatures to suppress microbial growth for a period of storage of 55 to 60 days. If these small scale experiments enable beef to be stored successfully for this period (sufficient to cover the duration of the voyage to Great Britain), the tests will be projected on to a semi-commercial basis.

The Handling, Freezing, and Storage of Edible Offal.—Investigations will be undertaken with the view to preventing the formation of "freezer and storage burn," and to secure, in the thawed product, a texture more closely resembling fresh, unfrozen offal.

The Freezing, Storage, and Thawing of Bacon Pigs.—Although experiments in England have indicated that it is possible to manufacture good bacon and hams from frozen pigs, there are several studies still needed to be carried out, more particularly in regard to the maximum possible duration of storage of the frozen pigs, the optimum temperatures of storage, and the best method of thawing. Studies in conjunction with one or several Departments of Agriculture, will be needed to determine the type of fat to be developed in the pig to withstand successfully the onset of rancidity during the chain of treatment of freezing, storage, thawing, curing, and smoking.

By-Products.—Further investigations on the rapid freezing of cuts of meat and on the treatment of endocrine glands and other organs for the subsequent manufacture of pharmaceutical preparations may be carried out in close co-operation with the Low Temperature Research Station, Cambridge, England.

General.—It is hoped that eventually the resources of the laboratory will permit of studies being undertaken of any phase of meat works technique, including physical investigations of the engineering side which, up to the present, has been developed almost entirely by empirical methods. It might also be mentioned that it is hoped

later to initiate experiments at this laboratory which may aid in the elimination of the wastage occurring in the transport and handling of tropical fruits, and also extend the range of tropical fruit exported from Queensland.

The Export of Australian Meat—A Trade Union's Gift for Research.

The bodies co-operating in the above note dealing with the investigations at Cannon Hill, Queensland, have recently received most welcome encouragement in the form of a spontaneous gift of £50 from the Queensland Branch of the Australasian Meat Industry Employees' Union. In forwarding a cheque for this sum, the branch secretary wrote as follows:—

> "In order to add our mite to furthering the efforts of your Council and to do our bit for the meat industry, I am directed to forward you the enclosed cheque for fifty pounds (£50), and to inform you that my Union will make a further contribution at a later late."

The Buffalo Fly Problem—Liberation of the Parasitic Wasp Spalangia sp.

The Council's Division of Economic Entomology hopes very shortly to liberate in North Australia a consignment of the parasitic wasp (*Spalangia* sp. (BzC))* with a view to ascertaining whether the widespread use of that variety of insect would be of value in the possible control of the buffalo fly.

As explained in the article, "The Buffalo Fly in Australia," which appeared in a previous issue of the *Journal* (Vol. 4, No. 4, 1931), it is recognized that no parasite can ever be expected to eradicate the fly completely, but nevertheless it is hoped that the introduction of the natural enemies of the fly will reduce its abundance to such an extent that it can no longer be considered as a serious menace to the cattle industry. Attention was first drawn to the possible value of *Spalangia* by Dr. Nieschulz, of the Veterinary Institute, Buitenzorg, Java. This reference was contained in a report made to the Council some years ago, the investigator mentioned then referring to the parasite as "BzC."

An abundance of evidence has been made available to the Council to the effect that the buffalo fly (*Lyperosia exigua* de Meijere), although it exists throughout the Netherlands East Indies, is not the pest there that it has become in North Australia. The importation of this wasp into Australia is not likely to be followed by any untoward results, as no species of the genus *Spalangia* is known to cause any economic damage, and the genus as a whole is to be regarded as comprising a group of beneficial insects. Some species have, in fact, already been found in Australia, and there is one in the Northern Territory which is known to parasitize puparia of *Lyperosia*. However, the species of which a hybrid race is now to be introduced is the most abundant and widespread so far discovered in the Netherlands Indies. It does not attack buffalo fly only, but is also parasitic on the puparia of other flies, e.g., *Musca, Biomyia,* &c. Nevertheless, it seems to prefer the buffalo fly.

The first district for the liberation work is to be Burnside Station, Brock's Creek, North Australia. Professor E. Handschin, of the

* Actually a hybrid race of *Spalangia* will be liberated, this hybrid being one of the crosses obtained from mating the Javanese species BzCL with the Australian species AC.

University of Basle, who has been in charge of the investigation in Java for the last two years, arrived in North Australia with a consignment of these insects in April last. As soon as it is quite certain that this shipment is free from secondary parasites, it is proposed to breed the wasps in numbers and to liberate them in the district.

The Buffalo Fly Problem—Co-operation of the Veterinary Services of the Netherlands East Indies.

With the transfer of Professor Handschin to Australia in order to make the liberation of *Spalangia* referred to in the previous note, the investigational work being carried out in the Netherlands East Indies by officers of the Council has been closed down, at any rate, for the time being.

Throughout their stay of some years in Java, the investigators have been freely afforded most helpful co-operation by the Veterinary Service of that country, and in particular have been accommodated at the State Veterinary Institute at Buitenzorg, where the whole resources of that Laboratory have been placed at their disposal.

With the closing down of the work, the Prime Minister has asked His Majesty's Consul-General at Batavia to convey to the Government of the Netherlands Indies the warm appreciation and thanks of the Commonwealth Government "for the advice and assistance which Dr. Van Eyck, as Director of Veterinary Services of the Netherlands Indies, Dr. C. Bubberman, as Director of the State Veterinary Institute, and their colleagues, Dr. B. J. Krijgsman and Mr. Pinto, have so helpfully and so ungrudgingly given to those officers in the service of the Commonwealth of Australia who have been pursuing investigations in Java into the problem presented by the buffalo fly."

A Sheep Branding Fluid Non-injurious to Wool.

(Contributed by D. Murnane, B.V.Sc., Division of Animal Health).

It is well known that the question of evolving a sheep branding mixture which meets the requirements of the wool-grower and at the same time is non-injurious to the wool (i.e., is easily removed in scouring operations) has been engaging the attention of the British Wool Industries Research Association for some time. The position has been more fully explained in a previous article (this *Journal*, February, 1931, p. 33), in which it was stated that the association recommends for use in Australia a mixture of the following composition:—

Wool fat	30	parts by weight
Resin	22	" " "
Carnauba wax ..	3	" " "
Kieselguhr ..	18	" " "
Ignited iron oxide ..	6	" " "
Emco spirit to desired consistency.		

While branding fluids made up to this formula are apparently quite satisfactory from the wool-scourer's point of view, they have been criticized in Australia on the ground of illegibility. That this criticism is not always warranted is made evident by the result of the following test.

In June, 1931, a number of shorn comeback wethers at the Veterinary Research Institute, Parkville—a portion of which is the Melbourne laboratory of the Division of Animal Health—were branded with a mixture made up according to the Association's formula given

above. Since branding, the sheep have been continuously exposed to the weather. Ten months have elapsed, and the brands are now plainly visible and equally as good as control brands on the same sheep made with a well known and commonly used black proprietary marking fluid.

Co-operative Horticultural Research—Root Stocks of Apples.

For some time past, certain problems have existed in connexion with the production of apples in Queensland, and particularly in the Stanthorpe district. Mr. R. G. Hatton, Director of the East Malling Research Station, who visited the district during his recent visit to Australia (see this *Journal*, Vol. 3, November, 1930, page 240), is of the opinion that the troubles are due very largely to the use of inappropriate root stocks. Incidentally, Mr. Hatton was rather impressed during his visit with the possibilities that existed in Australia for the application of the results of the researches that have been carried out under his direction at East Malling.

The matter has recently been discussed with the Manager of the Queensland Committee of Direction of Fruit Marketing, and as a result the Trustees of the Science and Industry Endowment Fund have arranged to appoint a research student to spend twelve months at the East Malling Station. After that, he will return to Australia, join the staff of the Council, and carry out some investigations at Stanthorpe. The Committee of Direction has agreed to meet the cost of his salary for the three years subsequent to his return to Australia.

The Part Played by Termites in the Destruction of Commercial Forest Trees—Report by Mr. G. F. Hill.

It has been suggested that there may be some association between heart rot and termite attack of ash trees (*Eucalyptus* spp.), and with the object of ascertaining whether this is the case, some preliminary investigations have been made in the Brindabella Mountains, Federal Capital Territory, by Mr. G. F. Hill, of the Division of Economic Entomology, Canberra. The results are recorded in a report recently received from the Division.

The evidence so far gathered does not support the view that there is any such association as that mentioned above; it was found, however, that many apparently healthy trees were rendered commercially valueless as a result of termite damage. Ninety-four per cent. of the larger trees (over 10 inches diameter) examined were found to harbour termites, whereas 4 per cent. only of the smaller trees (4 inches to 10 inches diameter) were infested. Five species of termites were found in ash trees (*E. gigantea* and *E. fastigata*), of which number only one, *Porotermes adamsoni*, is considered to be of major economic importance. (Fifty-eight per cent. of the large trees examined were found to be attacked by this species.) In most cases it appeared that the insects had gained access to the tree at a point near the ground where the trunk showed external evidence of fire damage. In all, about 450 living trees were examined in the F.C.T., of which number about 42 per cent. were found to be more or less damaged by the thirteen species of termites which were recognized. The habits of the more important species are discussed in the report, and photographs are appended to illustrate various types of damage.

The distribution of *Porotermes* coincides more or less closely with that of species of ash, which in New South Wales, Victoria, and

Tasmania are known to be seriously damaged by this termite. Further investigations are being made in the F.C.T., and it is suggested that these should be extended to include one of the principal ash forests of Victoria, where the losses due to "heart rot" are considerable.

Pine Aphis.—Liberation of Parasites.

In a previous issue (Vol. 4, November, 1931, page 254), reference was made to the damage caused to plantings of exotic pines (e.g., *Pinus radiata* (*insignis*)) in Australia by the pine Chermes* (*Pineus pini*). It was also stated that the Division of Economic Entomology was looking into the question of parasites likely to check the pest. Some progress has been made with that work, and recently a parcel of two varieties of insects, namely, *Hemerobius stigma* and *Leucopis obscura*, has been brought to Australia from England. Both these insects belong to a recognized class of entirely beneficial insects, and are incapable, from their structure, of doing any harm to economic plants since their larvae are predatory in insects, and are confined to Chermes and allies as hosts. For example, the larva of *L. obscura* is a legless grub which attacks Chermes and feeds upon them by piercing them and sucking them (or their eggs) of their contents. It grows rapidly, and when mature pupates beneath the woolly covering of its dead hosts.

After having been examined to be sure that they were true to name and free of parasites, the individuals of *Leucopis obscura* have been liberated on scale-infested trees in the Federal Capital Territory. Unfortunately, all the individuals of *Hemerobius stigma* died shortly after their arrival at Canberra. These particular individuals were sent in the adult stage, however, but within the next few weeks a further supply, this time consisting of eggs, will reach Australia.

Czecho-Slovak Academy of Agriculture.

Through the Consul-General for the Czecho-Slovak Republic, the Council has recently received a copy of a booklet describing the various activities of the Czecho-Slovak Academy of Agriculture. Arrangements are now being made for an interchange of literature between the two bodies.

The primary aim of the Academy, which was established in 1926, is the promotion of agricultural research and the practical application of its results in Czecho-Slovakia. Its activities are divided within the scope of six sections and of numerous working committees. The six sections already established are those of farming, forestry, horticulture, fruit growing and viticulture, agricultural industries, economics, and literature and culture.

The chief publication of the Academy is the C.A.A. Bulletin (Vestnik). Comprehensive summaries in English, French, and German are included in each issue, and give the gist of all of that number's contents likely to be of interest to foreigners.

The Academy is housed in a commodious building in Prague. Its management is in the hands of a Presidential Council, which is elected

* In the previous note this insect was referred to as the pine aphis (*Chermes pini*).

for a period of three years. It consists of a President, two Vice-presidents, and a General Secretary. Membership may be divided into three categories—

(1) Honorary membership awarded for permanent service to agriculture. The number of honorary members is limited to 40, half of whom may be foreigners.

(2) Corresponding membership. This is limited to foreign experts, and they are elected at the annual general meeting.

(3) Active membership is limited to Czecho-Slovak subjects who have done outstanding work in agricultural science, or who have contributed to rural cultural progress and agricultural production.

Recent Publications of the Council.

Since the last issue of the *Journal,* the following Bulletins and Pamphlets of the Council have been published:—

Bulletin No. 59.—"Radio Research Board: Report No. 2," by A. L. Green, M.Sc.

The investigations of the Radio Research Board have for their object the acquirement of knowledge of the propagation and characteristics of artificially and naturally generated electro-magnetic waves, with particular regard to those used in or affecting radio communication in Australia. The work described in Bulletin 59 has been carried out in pursuance of that object. The Bulletin itself consists of two papers, one entitled "The State of Polarization of Sky Waves," and the other "Height Measurements of the Heaviside Layer in the Early Morning."

The investigations have proved of no little interest in that they have confirmed an important hypothesis in connexion with the Heaviside Layer, namely, that whereas the polarization of a wave reaching the earth after reflection from the layer in the Northern Hemisphere has been found to be circular with the sense of rotation left-handed, the polarization of such a wave in the Southern Hemisphere would still be circular, but with the sense of rotation right-handed. No work on this particular matter had previously been ever carried out in the Southern Hemisphere. The investigations have also resulted in the discovery that one property of down-coming radio waves, namely, their polarization, is reasonably constant from night to night.

Bulletin No. 60.—"Radio Research Board: Report No. 3—The Influence of the Earth's Magnetic Field on the Polarization of Sky Waves," by W. G. Baker, B.E., B.Sc., and A. L. Green, M.Sc.

The Bulletin consists of a theoretical discussion of the earth's magnetic field on the propagation of sky waves of broadcast frequencies in the Heaviside Layer. The fundamental value of the report lies in the additional light it has thrown on the behaviour of radio transmissions once they have left the emitting station. Incidentally, it is interesting to note that authors predict that at night radio direction-finders in the Southern Hemisphere will be liable to much smaller errors of bearing when the direction-finder is situated to the north of the transmitter than when it is to the south.

Pamphlet No. 25.—"Termites (White Ants) in South-eastern Australia," by Gerald F. Hill.

The publication gives a simple method of identification of the varieties of white ants found in south-eastern Australia, and also a

discussion of the different ways these insects damage timber and forest trees. The pamphlet was written with the principal object of providing a reliable guide to foresters and others interested in the forest flora of Australia on the subject of the damage caused by termites or white ants. A further objective of the paper is to interest a large number of forestry workers in these insects, in the hope that they will collect them more frequently and send in their specimens to Mr. Hill for identification. With these ends in view, the paper has been written in simple language, and such scientific and technical terms as are unavoidably used have been defined in a glossary.

Pamphlet No. 26.—"The Irrigation of Horticultural Community Settlements," by A. V. Lyon, M.Agr.Sc.

This pamphlet was prepared largely at the instance of a representative conference on the dried fruit industry, which met late in 1930, and which considered that one way in which the industry could be helped would be by assisting the various Irrigation Advisory Boards, firstly, by furnishing them with technical advice concerning the periodicity of irrigation; and, secondly, by carrying out a programme of investigational work in regard to frequency and method of irrigation as affected by soil type, climate, and crop requirements. The publication, which was prepared mainly for the guidance of Advisory Boards in the Murray Valley settlements, presents data indicating the nature of the main controlling factors leading to possible waste at the present time. It also contains suggestions regarding the initiation of corrective measures.

Forthcoming Publications of the Council.

The following publications of the Council are now in the press:—

Bulletin No. .—"Studies in Supplementary Feeding of Merino Sheep for Wool Production, I." By Hedley R. Marston.

Bulletin No. .—"The Ripening and Transport of Bananas in Australia." By W. J. Young, D.Sc., L. S. Bagster, D.Sc., E. W. Hicks, B.A., B.Sc., and F. E. Huelin, B.Sc.; and in part by R. A. Holloway, B.Sc., B.E., and O. P. Barr, B.E., of the New South Wales Government Railways.

Bulletin No. .—"Radio Research Board: Report No. 4." 1. A Preliminary Investigation of Fading in New South Wales, by A. L. Green, M.Sc., and W. G. Baker, B.E., B.Sc. 2. Studies of Fading in Victoria: A preliminary Study of Fading on Melium Wavelengths at Short Distances, by R. O. Cherry, M.Sc., and D. F. Martyn, Ph.D., A.R.C.Sc. 3. Studies of fading in Victoria: Observations on Distant Stations in which no Ground Wave is Received, by R. O. Cherry, M.Sc.

Bulletin No. .—"A Soil Survey of the Cadell Irrigation Area and New Era, South Australia." By T. J. Marshall, B.Sc. (Agr.), and A. J. King, A.A.C.I.

Pamphlet No. .—"The Possibilities of the Zebu Cross in connexion with the Cattle Industry of North Australia." By R. B. Kelley, B.V.Sc.

Pamphlet No. .—"The Pig Industry. Report on observations in Great Britain and America with possible Australian Applications." By R. B. Kelley, B.V.Sc.

H. J. GREEN
GOVERNMENT PRINTER
MELBOURNE